MEDICAL LIBRARY

APR 10 1950

CANCER RESEARCH

VOL. 10 APRIL 1950 No. 4

CONTENTS

SCIENTIFIC PROCEEDINGS

AMERICAN ASSOCIATION *for* CANCER RESEARCH, INC.

Atlantic City, April 16-18 1950

Abstracts 201

Author Index 252

Announcement 254

THE OFFICIAL ORGAN OF THE

AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

Published by THE UNIVERSITY OF CHICAGO PRESS

CANCER RESEARCH

This journal is sponsored by The American Association for Cancer Research, Inc.; The Anna Fuller Fund; Cancer Research Division, Donner Foundation, Inc.; The Jane Coffin Childs Memorial Fund for Medical Research; The Elsa U. Pardee Foundation; and The American Cancer Society.

HAROLD P. RUSCH, *Editor-in-Chief*

Editorial Advisory Board

JOSEPH C. AUB
CARL A. BAUMANN
JOHN J. BITTNER
AUSTIN M. BRUES
E. V. COWDRY
HUGH J. CREECH
M. DEMEREC
F. DURAN-REYNALS
JACOB FURTH
W. U. GARDNER
SAMUEL GRAFF
HARRY S. N. GREENE
JESSE P. GREENSTEIN
CHARLES C. HUGGINS
ALBERT L. LEHNINGER
C. C. LITTLE
BALDUIN LUCKÉ
G. BURROUGHS MIDER
JAMES A. MILLER
C. P. RHOADS
A. J. RIKER
MURRAY J. SHEAR
MICHAEL B. SHIMKIN
PAUL E. STEINER
ROBERT E. STOWELL
ALBERT TANNENBAUM
SHIELDS WARREN
ARNOLD D. WELCH

CANCER RESEARCH is published monthly for Cancer Research, Inc., by the University of Chicago Press, 5750 Ellis Avenue, Chicago 37, Illinois. The subscription price is $12.50 per year; the price of single copies is $1.25. Orders for less than a full year will be charged at the single copy rate. Postage is prepaid by the publishers on all orders from the United States and its possessions. No extra charge for countries in the Pan American Postal Union. Postage is charged extra as follows: For Canada and Newfoundland, 48 cents on annual subscriptions (total $12.98), on single copies 4 cents (total $1.29); for all other countries in the Postal Union, $1.20 on annual subscriptions (total $13.70), on single copies 10 cents (total $1.35).

The following is an authorized agent:

For the British Empire, except North America and Australasia:

Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1, England. Prices of yearly subscriptions and of single copies may be had on application.

Business communications, remittances (in United States currency or its equivalent), and subscriptions should be addressed to THE UNIVERSITY OF CHICAGO PRESS, 5750 Ellis Avenue, Chicago 37, Illinois. All other communications should be addressed to Harold P. Rusch, M.D., McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wisconsin.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

No responsibility is accepted by the Editors, by Cancer Research, Inc., or by the publishers of *CANCER RESEARCH* for opinions expressed by contributors.

NOTICE TO SUBSCRIBERS: If you change your address, please notify us and your local postmaster immediately. The Post Office does not forward second-class mail.

Entered as second-class matter, February 15, 1949, at the post office at Chicago, Ill., under the Act of March 3, 1879.

Copyright 1950 by Cancer Research, Inc.

CANCER RESEARCH

VOLUME 10 APRIL 1950 NUMBER 4

SCIENTIFIC PROCEEDINGS

AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

ATLANTIC CITY

APRIL 16–18, 1950

ACID-SOLUBLE, LIPID, AND PROTEIN PHOSPHORUS IN NORMAL, PREGNANCY STIMULATED, AND CANCEROUS MAMMARY GLANDS OF INBRED MICE.

S. ALBERT, RALPH M. JOHNSON (by invitation), and MARJORIE S. COHAN (by invitation). (Richard Cohn Radiobiology Laboratory, Detroit Institute of Cancer Research, Detroit 1, Mich.)

Studies on the distribution and metabolism of phosphorus in various fractions of mouse mammary tissue and mammary carcinomas have been continued. Normal and pregnant female mice of high and low cancer strains and mice with spontaneous mammary carcinomas were injected with radioactive phosphorus and killed at different time intervals after injection. Each mammary gland or mammary cancer was separated into various phosphorus-containing fractions; nitrogen, phosphorus, and radioactivity determinations were made on each fraction.

Total phosphorus, expressed on a basis of weight of wet tissue, of the acid-soluble, lipid, and phosphoprotein fractions did not differ appreciably between the resting and pregnancy-stimulated glands. In contrast, however, there was considerably more phosphorus in the same three fractions isolated from tumors. The lipid fraction of the tumors differed from that of the normal and stimulated mammary glands, in that a lower P/N ratio was found in the former than in that of the nontumorous glands.

Radioactivity determinations revealed a rapid uptake of P^{32} in the acid-soluble and phosphoprotein fractions of all tissues studied, while the lipid fractions accumulated phosphorus relatively slowly. The lipid fractions from tumors accumulated more P^{32} than did the corresponding fractions of either the resting or stimulated mammary glands. The phosphoprotein fractions of all tissues studied, while containing relatively little phosphorus, appeared to be one of the most active in accumulating P^{32}.

DEMONSTRATION OF MILK FACTOR IN A C3H MOUSE MAMMARY TUMOR AFTER THE TUMOR HAD BEEN TRANSFERRED 31 TIMES IN FERTILE EGGS.

M. I. ARMSTRONG (by invitation) and A. W. HAM. (Department of Anatomy, University of Toronto, Toronto, Can.)

A spontaneous mammary tumor from a C3H female mouse was cultivated in the yolk sacs of fertile eggs. On its serial transfer in this medium it developed enhanced lethal properties for chick embryos; this required that the tumors be harvested on the thirteenth day of incubation. After 31 transfers, 2 cc. of tumor tissue, harvested from three eggs, was diluted to 20 cc. with fluids from the eggs. The mixture was then frozen in a carbon dioxide refrigerator after which it was thawed under the cold water tap. This procedure was carried out 3 times. The mixture was then diluted further to 2,000 cc. with 0.85 per cent saline. Thirty test mice, the 34–41-day-old female offspring of C3H males and C57 black females, were then each injected with 0.5 cc. of the mixture. Thirty uninjected litter mates of the injected mice served as controls. All the mice were bred regularly until they were 2 years old, when the experiment was terminated.

Of the 30 mice injected with the diluted thrice-

frozen and thawed mixture, twelve developed mammary tumors when between 10 months and 2 years of age. None of the uninjected control mice developed mammary tumors.

USE OF CHROMATOGRAPHIC TECHNICS IN THE STUDY OF METABOLITES OF HUMAN TUMORS. Jorge Awapara (introduced by R. Lee Clark, Jr.). (Anderson Hospital for Cancer Research, University of Texas, Tex.)

Malignant tissues have been the object of continuous investigations, which were carried out in the hope of detecting some biochemical difference from their normal counterparts. The classical technics of analytical chemistry have revealed nothing, and in cases where outstanding differences were observed the discrepancy between various laboratories has been great. The development of more refined technics has made it possible to initiate a new attack on the old problem; chromatographic technics have been applied already in several instances, and the measurement of metabolites in tumors has been started. At least, the confusion which exists today in the literature will be clarified. Ethanolaminophosphoric ester has been claimed to be a characteristic metabolite in tumors. Data obtained in our laboratories using the more sensitive chromatographic analysis have shown definitely that the phosphatide in question is present in nearly every organ of the rat. The system used on the study of this particular metabolite is now being extended to other compounds.

SOME CHANGES IN AMINO ACID METABOLISM ASSOCIATED WITH CHRONIC CALORIC RESTRICTION. A. D. Barton (by invitation), R. K. Boutwell, and H. P. Rusch. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

Although the marked inhibitory effect of caloric restriction on the formation of experimental tumors is well established, little is known concerning the mechanism by which this occurs. In order to investigate the effect of caloric restriction on amino acid and protein metabolism, experiments employing amino acids labeled with C^{14} have been undertaken. The present report deals with differences in the rate of excretion of radioactive CO_2 in the breath following intravenous injection of glycine-α-C^{14} and tryptophane-β-C^{14} into female, Rockland strain mice. The observed rates are believed a measure of the rate at which the labeled amino acid is deaminated, since (at least in the case of glycine) it appears that these rates are approximately 100-fold lower than those observed when carbohydrate or fatty acid intermediates of similar molecular weight are administered.

When the labeled glycine (50–300γ; 0.5–3μc/100 gm) or tryptophane (400–1,200γ; 0.5–1.5μc/100 gm) was administered from 1 to 2 hours after feeding, the rate of deamination in the mice on a restricted diet was about twice as great as that in the mice on an *ad libitum* diet. However, the two rates were approximately the same when the labeled amino acids were administered 14 hours after feeding. Adrenalectomy caused a reduction in the rate of deamination which was not restored completely by administration of adrenal cortical extracts. Experiments designed to determine the extent to which the observed variations are dependent upon variations in the glycine metabolic "pool" are now in progress.

LETHAL AND TUMOR-DAMAGING EFFECTS OF CERTAIN TRIVALENT ARSENICALS, AS MODIFIED BY 2,3-DIMERCAPTOPROPANOL (BAL), AND BY ADRENAL EXTRACTS. Lyle V. Beck. (National Cancer Institute, Bethesda 14, Md.)

Normal (nontumor-bearing) mice were partially or completely protected against a subcutaneous LD 60–100 dose of each of three tumor-damaging trivalent arsenicals by the intraperitoneal injection of BAL (at least 1 molecule of BAL per molecule of arsenical). For mapharsen (Compound 192), measurable protection was secured by injecting BAL 15 and 30 minutes, but not zero and 16 minutes, after the arsenical. For 3,3′-diamino-6, 6′-dimethoxyarsenobenzene-N, N′-dimethylene-disodium sulfonate (Compound 828), maximum protection was secured by injecting BAL 1–3 hours after the arsenical. For arsenious acid (Compound 2174), maximum protection was secured by injecting BAL 1–2 hours after the arsenical. Appreciable reduction in arsenious acid-induced mortality was also secured by injection of Upjohn Co. adrenal extract. Maximum protection was secured with the beef extract upon injection about 1 hour before, and with the hog extract upon injection about 2 hours before the arsenical.

In mice bearing Sarcoma 37, compounds 192, 828, and 2174 produced damage to the tumors upon subcutaneous injection of an approximately LD 5 dose. When BAL was also administered, counteraction of damage induced in the tumor was easily detected only when the same time interval between arsenical and BAL was employed as had been found to give the maximum reduction in mortality in nontumor-bearing mice.

THE INFLUENCE OF CORTISONE (COMPOUND E) UPON THE GROWTH OF A TRANSPLANTED RHABDOMYOSARCOMA IN C3H MICE. GEORGE M. HIGGINS (by invitation), KATHRYN A. WOODS (by invitation), and WARREN A. BENNETT. (Mayo Foundation, Rochester, Minn.)

The observation that 11-dehydro-17-hydroxycorticosterone (Compound E) inhibited the growth of a highly malignant tumor implanted subcutaneously into young mice and caused a rapid regression of well developed tumors (Heilman and Kendall, 1944) prompted a study of the influence of cortisone upon the growth of certain other transplantable tumors.

This report embodies the preliminary results of our study of the influence of this hormone upon the growth of a rhabdomyosarcoma transplanted by trocar into subcutaneous tissues of C3H mice. A donor, of the 63rd transplant generation, was provided for our use by Dr. Elizabeth Green of the Institute for Cancer Research. We are indebted to her for her co-operation and interest in our study.

Twenty adult mice were implanted with a fragment of tumor tissue. Ten of these constituted the test group, and on the day following the implantation each mouse received 1.0 mg. of cortisone, intramuscularly. Ten mice served as controls and were uninjected. Tumors grew rapidly in all mice of the control group; but growth was completely inhibited in mice which received cortisone daily. Eight days after implantation cortisone was discontinued for the next 7 days. Growth of the implant then took place, and continued until cortisone therapy was re-instituted. Further growth of the tumors was restricted, but they did not appear to regress. Photographs of the mice and sections of the tumors in treated and untreated mice will be shown.

THE DISAPPEARANCE OF LEUKEMIC CELLS IN NONLEUKEMIC RECIPIENTS DURING TRANSFUSIONS AND CROSS-CIRCULATION STUDIES. HOWARD R. BIERMAN, R. L. BYRON, JR. (by invitation), and JONATHAN T. LANMAN (by invitation). (The Laboratory of Experimental Oncology, National Cancer Institute, and the University of California Medical School, San Francisco, Calif.)

Rapid transfusions of 9–120 billion leukemic leukocytes into nonleukemic subjects revealed that the leukemic leukocytes fail to appear on the arterial side of the lesser circulation. Heparinization permits leukemic cells to pass the usual removal mechanism in the lungs.

By developing a method of direct artery-to-artery cross-circulation in man an attempt was made to filter out leukemic cells in leukemic patients in the lung filter of nonleukemic patients. Seven cross-transfusions have been completed on volunteer patients with various neoplastic diseases. It is possible to cross-transfuse 6–10 l. of blood per hour both to and from the donor and recipient. To date, 150 l. of blood have been cross-transfused within a 26-hour period. Marked decreases in leukocyte counts in the leukemic patients were observed some hours *following* the cross-transfusions. Leukemic skin infiltrations disappeared overnight, and clinical improvement was noted within an hour after the cross-circulation was begun. No evidence of leukemia has been detected in any recipient. Postmortem studies showed degenerating leukocytes and absence of leukemic infiltration of the liver and spleen and bone marrow of the leukemic patients. The procedure is experimental, and extreme caution must be exercised regarding blood type and Rh compatibility, nondeliberate transmission of disease, and both immediate and late effects of immunological reactions.

The concept that an impaired removal mechanism may be as important as proliferation in leukemia has been strengthened.

THE EFFECT OF VIRUS AND BACTERIAL INFECTIONS ON NEOPLASTIC DISEASES. HOWARD R. BIERMAN, WILLIAM MCD. HAMMON (by invitation), BERNICE U. EDDIE (by invitation), KARL F. MEYER (by invitation), and MICHAEL B. SHIMKIN. (Laboratory of Experimental Oncology, National Cancer Institute, and the University of California Medical School, San Francisco, Calif.)

The virus of *lymphopathia venereum* has been given intravenously to five patients with metastatic neoplastic diseases. Five patients with lymphoma or lymphatic leukemia have been observed following the intramuscular and intracutaneous inoculation with the virus of feline agranulocytosis. Changes in blood chemistry, hematology, and clinical status were noted. A variety of skin eruptions was noted with the cat leukopenic virus. Marked but temporary clinical and hematologic remissions of 3 weeks to 6 months have been observed with varicella infections in three children with acute lymphogenous leukemia.

Marked hematologic changes leading to temporary aplasia of the marrow have been observed with massive hemolytic staphylococcus aureus infections in acute lymphatic leukemia.

A variety of interesting observations have been

made in 16 cases, but in no case was there noted any permanent beneficial effect as regards the neoplastic process.

TISSUE CULTURE STUDIES WITH 2,6-DIAMINOPURINE AND RELATED SUBSTANCES. JOHN J. BIESELE, RUTH E. BERGER (by invitation), and GEORGE H. HITCHINGS (by invitation). (Sloan-Kettering Institute for Cancer Research, New York 21, N.Y., and the Wellcome Research Laboratories, Tuckahoe, N.Y.)

2,6-Diaminopurine selectively damaged tissue cultures of the mouse sarcomas, Crocker 180, T241, and Ma387, at concentrations causing little or no damage to cultures of embryonic mouse skin or heart cells. However, embryonic rat skin cultures proved more sensitive to this purine than rat sarcoma R39 or Walker carcinosarcoma 256.

The sensitivity to diaminopurine was not related to the mitotic growth rate at the time of administration.

The damage from 2,6-diaminopurine could be prevented by simultaneous administration of adenine, with the proportion of adenine to diaminopurine being less, the lower the sensitivity of the tissue to diaminopurine.

Little or no protection from diaminopurine was given by hypoxanthine, xanthine, guanine, isoguanine riboside (itself toxic), adenosine, muscle or yeast adenylic acids, guanylic acid, glycine, or folic acid. The last damaged Sarcoma 180 and T241 *in vitro*.

RECOVERY OF THE MAMMARY TUMOR MILK AGENT FOLLOWING TRANSFER BY THE MALE PARENT. JOHN J. BITTNER. (Division of Cancer Biology, Department of Physiology, University of Minnesota Medical School, Minneapolis 14, Minn.)

Confirming previous observations of Andervont, data have been obtained by crossing females of the C strain with Z or C3H males, that the paternal parent may transfer the milk agent. None of the C females used by Andervont developed mammary tumors; the milk agent was demonstrated by breeding tests, but numerous tumors gave negative assays when tested.

Four of the 8 C females that were used gave rise to tumors, although the controls have remained noncancerous. From the incidence in the CZF_1 progeny with cancerous C mothers, the approximate time of "infection" could be determined. Prior to infection, the incidence was low; after infection the incidence was approximately 90 per cent. Extracts of three tumors (two from C females) were tested and were found to have the milk agent. The hybrids born to the noncancerous C females had a low incidence. Comparable data from other crosses will be presented, and also additional evidence that mice born of parents neither of which possess the milk agent may develop mammary cancer and have the agent.

The amount of the milk agent injected in an extract of 10^{-3} gm. equiv. of spontaneous mammary cancer may be transferred in the milk to the progeny born in the first litter.

The milk agent could not be demonstrated in the extract of a mammary carcinoma with extensive squamous metaplasia.

Several surveys are being made on the genesis of human breast cancer to determine if there might be a nursing influence as a causative factor. If one is active, the observations on mice that it could either be transferred by the male parent or be obtained through a blood transfusion could make the interpretation of the human data most difficult.

TISSUE RESPIRATION STUDIES AND TUMOR VISUALIZATION BY TRIPHENYL TETRAZOLIUM CHLORIDE. MAURICE M. BLACK, I. S. KLEINER (by invitation), and F. D. SPEER (by invitation). (New York Medical College, New York, N.Y.)

Triphenyl tetrazolium chloride is reduced enzymatically to a water-insoluble red-colored formazan in the cytoplasm of viable normal and tumor tissue. Such reductase activity may be measured quantitatively in terms of color units/mg tissue/hr, thus providing an expression of the endogenous dehydrogenase activity of the tissue. The technic may also be applied to studies of substrates and inhibitor effects in a simple and reproducible manner.

Kidney, liver, and epithelial surfaces, in general, possess high dehydrogenase activity in contrast to spleen, lymphoid tissue, fibrous tissue, and inflammatory granulomata, which reduce the dye to a lesser degree. Carcinomata tend to retain the high dehydrogenase activity of the tissue of origin in the primary tumor and in the metastatic spread. Reticulum cell lymphosarcoma and lymph nodes of acute myeloblastic leukemia reduce the dye actively in contrast to the nodes of Hodgkins disease and chronic lymphatic leukemia.

The high reductase activity of carcinomatous tissue makes possible the ready visualization of invasion into the poorly staining fibrous and lymphoid tissues. This finds application as an aid to frozen section examination in the rapid diagnosis and demonstration of human cancer.

Triphenyl tetrazolium chloride when used in the

manner indicated constitutes a valuable and economical tool in the study of tissue respiration and the gross demonstration of the spread of tumors.

THE BASIC PROTEINS OF THE CELL NUCLEUS. WALTER L. BLOOM (by invitation), BETTY CODGELL (by invitation), and GEORGE T. LEWIS. (Department of Biochemistry, School of Medicine, Emory University, Ga.)

The basic proteins of cell nuclei have been isolated from calf thymus by an adaptation of the method of Kossel, which utilizes a water extraction for the isolation of histone. Material so isolated has been subjected to fractionation, primarily by isoelectric precipitation. Two fractions have been obtained which are readily reproducible in successive preparations. Fraction I precipitates at pH 10, while Fraction II remains in solution in the basic medium. Lyophilization of the supernatant liquid from Fraction I gives Fraction II. Fraction I comprises approximately 60 per cent of the total protein isolated.

Fraction I contains tryptophane (approximately 0.65 per cent), whereas Fraction II is free from this amino acid. The previously water-soluble material precipitated at pH 10 apparently undergoes rapid denaturation, because it is subsequently soluble only in acid. Electrophoretic studies are compatible with the presence of only two fractions in the isolated protein. Nitrogen content of the protein before fractionation is approximately 13 per cent.

Previous investigators have obtained fractions which may or may not be identical with these. It would appear that the method of preparation determines the structure of the product. This will be discussed from the standpoint of the content of basic amino acids, particularly arginine and lysine.

The suggestion has been made that the material corresponding to Fraction I is the protein of the chromosome. Such preparations might be expected to have a marked influence on cell division and, hence, tissue growth.

LIPIDS OF THE ADRENALS AND BLOOD PLASMA IN CANCER. W. R. BLOOR, FRANCES L. HAVEN, and ETHEL ASHWORTH (by invitation). (University of Rochester School of Medicine and Dentistry, Rochester, N.Y.)

The lipid content of the blood plasma and adrenals has been investigated in rats bearing Walker tumor 256, in rats in which the transplanted tumor did not grow, in rats in which the tumor regressed, and in two groups of normal rats. Values for total lipid of the blood plasma were much higher than normal for the tumor-bearing group, slightly higher than normal for the regressors, and normal for the "non-takes." Values for plasma cholesterol of all groups were within the normal range.

In the adrenals of rats with tumors, values for total steroids were much below normal; the content of the adrenal in total steroid was inversely proportional to the size of the tumor expressed as percentage of the total body weight. Regressors and "non-takes" had values somewhat higher than normal for total steroid of the adrenals. Pronounced decreases in steroids other than cholesterol accounted for the reduction in total steroids of the adrenals in the tumor-bearing group.

STUDIES ON THE EFFECT OF LOW TEMPERATURES ON THE TRANSPLANTABILITY OF NORMAL AND NEOPLASTIC TISSUE. HERMAN T. BLUMENTHAL, LAWRENCE B. WALSH (by invitation), and DONALD GREIFF (by invitation). (Departments of Pathology and Biology, St. Louis University, St. Louis 4, Mo.)

Gye, Mann, and co-workers have recently reported the successful passage of frozen and frozen-dehydrated tumor material in mice. They have interpreted these findings as indicating that tumor cells do not survive this treatment and that these observations constitute proof of the passage of a virus. The investigations reported here show that not only tumor cells but also normal tissues may survive freezing to as low as $-190°$ C. and may be successfully transplanted. The tumor used was Sarcoma 37 of mice; the normal organ used was the thyroid gland of the guinea pig, which was successfully autotransplanted after refrigeration. The latter findings do not disprove the possibility of a viral transmission of neoplastic disease but rather prove that the criteria used for determining whether or not this mode of transmission obtains must be more strictly defined.

INDUCTION OF ACCESSORY LIMBS IN SALAMANDERS WITH MIXTURES CONTAINING CARCINOGENS. CHARLES BREEDIS. (Department of Pathology, University of Pennsylvania, School of Medicine, Philadelphia 4, Pa.)

It is known that salamanders may develop accessory limbs or limb parts following certain surgical procedures, most frequently after transplantation of limb buds or deviation of the course of nerves. In the present experiments, performed in an attempt to induce neoplasia, similar growths

developed after the injection of some substances but not of others.

Substances possibly carcinogenic for salamanders were introduced by means of a single injection into one forelimb of adult *Triturus viridescens*, employing a total of 420 animals. Coal tar, alone, or with added carcinogens, produced a high incidence of the growths (20–40 per cent). Of 44 animals receiving methylcholanthrene in olive oil, one developed an accessory limb. Acetylaminofluorene or Scharlach R in olive oil, benzpyrene in vaseline, vaseline alone, or fragments of kidney carcinoma of the frog produced no growths. The accessory limbs and limb parts developed from the injected site after 50–100 days, grew rapidly at first, and ceased growing at approximately 200 days. They varied from almost perfect reduplications of distal portions of the forelimb to flipper-like and digit-like projections. Attempts to transplant them to the body wall were unsuccessful. Microscopic examinations showed no evidence of malignancy.

Whether carcinogens present in tar were responsible for its marked activity is unknown. Its action appears to be more than a nonspecific necrotizing effect, since methylcholanthrene and Scharlach R produced as much or more necrosis.

ISOLATION AND MORPHOLOGY OF MAMMALIAN CHROMOSOMES. Austin M. Brues and Leola Rietz (by invitation). (Argonne National Laboratory, Chicago, Ill.)

Chromosome-like structures have been isolated from a variety of mammalian tissues and tumors, including leukemic blood cells. Examination of nuclei partly disintegrated by the colloid mill makes it appear that these structures are preformed within the nucleus and are extruded as a result of the action of the mill. As shown previously by other investigators, their staining reactions are those of chromosomes. Many of these structures are seen lying in pairs, which may be separated through part of their length. Electron micrographs of fixed preparations show an occasional rope-like structure; these forms have been seen in conjunction with more usual types in partially disintegrated nuclei. Under proper conditions of fixation, the chromosomes appear to be surrounded by a thin film. Measurements of chromosomes in electron micrographs give a median volume of the order of 0.5 μ^3 and lengths vary between 3 and 20 μ. Those examined by phase contrast in wet unfixed preparations show a similar range of lengths, but their volumes are somewhat greater. Examined in this way in the fresh state, constricted bands of increased density are seen, which respond differentially to certain agents.

THE EFFECT OF LOCAL X-RAY IRRADIATION UPON THE DEVELOPMENT OF VARIOUS PARTS OF THE BODY OF THE YOUNG MOUSE. V. V. Brunst (by invitation), D. J. Barnett (by invitation), and Frank H. J. Figge. (Departments of Anatomy and Radiology, University of Maryland Medical School, Baltimore 1, Md.)

The first part of the film demonstrates the method of local irradiation of 2-day-old mice of the C57 (black) strain.

The second part demonstrates the effect of local irradiation. In addition to the other effects, all irradiations resulted in temporary inhibition of hair development. After some time, growth of nonpigmented hair was observed. The irradiation of the middle part of the body, in many cases, paralyzed the posterior limbs and the tail. Obviously, this is a result of partial inhibition of vertebral column growth which gives rise to compression of the spinal cord. Irradiation of the head inhibited the development of the treated region which caused a general disturbance of the animals' movements. Irradiation of the posterior limbs and the tail permanently inhibited the growth of treated parts.

STIMULATING EFFECT OF X-RAY IRRADIATION ON THE TAIL DEVELOPMENT OF YOUNG AXOLOTL (*SIREDON PISCIFORMIS*). V. V. Brunst (by invitation), E. A. Sheremetieva-Brunst (by invitation), D. J. Barnett (by invitation), and Frank H. J. Figge. (Departments of Anatomy and Radiology, University of Maryland Medical School, Baltimore 1, Md.)

Local x-radiation of axolotl tails caused inhibition of growth of the treated region, and, in many, it gave a stimulus for a new development. The beginning of this new tail formation was observed 70–75 days after irradiation. The histological investigation revealed this first sign of new development. The formation of the first cartilaginous skeletal element was observed during the acute primary reaction to irradiation (about 50 days after irradiation). Usually, this new tail formed from the dorsal and more proximal portion of the original tail. This proximal portion consisted also of treated tissues, but those treated with a weak dose (boundary zone—"zone of stimulation"). The new tail always has a cartilaginous skeleton. A normal animal of this age has only a notochord in the distal part of the tail. This investigation shows that the development of a secondary tail after local roentgen irradiation is a result of the roentgen-stimulation effect. The new tail development after local roentgen irradiation is an example of a pro-

found change in the process of ontogenic development induced by x-ray.

STUDIES ON THE EFFECTS *IN VITRO* OF X-RADIATION ON THE AGENT OF CHICKEN TUMOR I (ROUS SARCOMA). W. Ray Bryan, Egon Lorenz, and John B. Moloney (by invitation). (National Cancer Institute, National Institutes of Health, Bethesda 14, Md.)

Determinations were made of the dose-survival curves following irradiation with x-radiation in six experiments carried out with quantitative bioassay technics and involving different methods of preparation of suspensions of the agent of chicken tumor I. The nature of the curve was found to vary with the method of preparation and with the associated varying proportions of extractable tumor tissue constituents which were separable by differential centrifugation.

The interpretations of the results are discussed.

THE TURNOVER OF C^{14}-LABELED ALANINE AND GLYCINE IN THE PROTEINS OF RAT LIVER. Nancy L. R. Bucher and Ivan D. Frantz, Jr. (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Massachusetts General Hospital, Boston, Mass.)

An investigation of amino acid turnover in the proteins of rat liver under various conditions was undertaken on the assumption that the growth capacity of a tissue is closely related to its ability to build proteins. In preliminary experiments, it was found that the half-life of radioactivity in liver proteins which had been tagged with C^{14}-labeled glycine could be reduced by more than half by the repeated administration of large amounts of nonradioactive glycine. Glycine-labeled controls "washed out" with repeated injections of dl-alanine showed no significant decrease in the half-life of radioactivity in their liver proteins.

On the other hand, when liver protein was tagged with C^{14}-labeled alanine, the half-life, even in the absence of "washing out," was 60 per cent less than with labeled glycine. Administration of nonradioactive alanine failed to alter this turnover rate, but a definite decrease in half-life again followed "washing out" with glycine.

The rate of loss of activity in alanine-labeled liver protein thus appeared to be significantly faster than that in glycine-labeled protein. Under the conditions of these experiments, "washing out" the rat by maintaining a high blood level of nonradioactive alanine did not alter the half-life, but "washing out" with glycine reduced it considerably, regardless of whether the protein was initially labeled with glycine or alanine.

REGENERATION OF THE LIVER IN PARABIOTIC RATS. Nancy L. R. Bucher, J. F. Scott, and J. C. Aub. (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Massachusetts General Hospital, Boston, Mass.)

Relatively little is known about factors that regulate the basic mitosis rates of various tissues. Mitoses are extremely rare in the liver of the normal adult rat—approximately one in 34,000 cells. Following extirpation of $\frac{2}{3}$ of the liver, however, active cell division occurs. We have attempted to determine whether factors circulating in the blood stream during regeneration could serve to initiate this proliferative process.

Rats were joined in parabiosis at from 1 to 3 months of age. At 2–7 months later, one partner was partially hepatectomized, and at intervals of 24, 48 or 72 hours afterwards both partners were autopsied. The intact liver of the nonhepatectomized partner was examined for alterations known to be characteristic of regenerating liver.

Mitosis counts were within normal limits at 24 hours, but by 48 and 72 hours the mean value was 0.0306 per cent for seven nonhepatectomized partners, as compared to 0.0048 per cent for thirteen normal controls. The difference seems to be significant. (Either 30 mitoses or 100,000 nuclei were counted.)

Alkaline phosphatase and DNA determinations were not significantly different from normal: RNA showed a suggestive increase ($p < 0.05$).

No increase in mitosis occurred in the livers of four normal rats following a small hepatic biopsy; hence the above result was not attributable to the operative procedure per se.

These findings suggest that there may be a blood-borne mechanism for control of liver mitosis.

INHIBITION OF THE CROCKER MOUSE SARCOMA 180 BY CERTAIN ETHYLENEIMINE DERIVATIVES AND RELATED COMPOUNDS. S. M. Buckley (by invitation), C. C. Stock, M. L. Crossley (by invitation), and C. P. Rhoads. (Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City, and the Calco Division of the American Cyanamid Company.)

Because of their chemically highly reactive nature, certain ethyleneimine derivatives and related

compounds were tested in a screening program employing the Crocker mouse sarcoma 180.

Groups of five mice (20 ± 2 gm., white Swiss) were implanted subcutaneously with Sarcoma 180. Starting 24 hours later, the chemicals were administered daily by intraperitoneal injections for 7 days. Repeated intraperitoneal injections of 2,4,6-tris (1-aziridyl) imino-*s*-triazine (0.75 mg/k) and 2-amino-4,6-bis (1-aziridyl)-*s*-triazine (0.75 mg/k) caused a marked inhibitory effect upon the tumor development as judged by comparison of tumor size in treated and untreated groups of animals at the end of 1 week. These effects were achieved at levels of moderate toxicity for there was considerable relative weight loss; however, inhibition was observed at levels as low as 0.25 mg/k without significant weight loss.

N^2, N^2, N^4, N^4, N^6, N^6-hexamethyl melamine and N, N^1-(2,3-tolylene)-bis (1-aziridine carboxamide) also showed a slight but definite inhibition. N^2, N^2-ethylenemelamine and melamine gave negative results. In addition, N^2, N^4, N^6-tris(hydroxymethyl)-melamine, N-octadecyl-1-aziridinecarboxamide and N, N^1-hexamethylene-bis (1-aziridine carboxamide) are among related compounds tested.

THE PREVENTION OF THE ANTI-LEUKEMIC EFFECT OF 4-AMINO-N^{10} METHYL PTEROYLGLUTAMIC ACID BY CITROVORUM FACTOR.

Joseph H. Burchenal, S. F. Johnston (by invitation), H. P. Broquist (by invitation), and T. H. Jukes (by invitation). (Division of Chemotherapy of the Sloan-Kettering Institute, New York City, and the Lederle Laboratories Division of the American Cyanamid Company, Pearl River, N.Y.)

It has previously been shown that the chemotherapeutic effect of 4 amino N^{10} methyl pteroylglutamic acid in prolonging the survival time of mice with transmitted leukemia can be prevented by prior administration of massive doses of pteroylglutamic acid. Since the citrovorum factor (CF) has been shown to prevent the toxicity of 4 amino PGA in the bacterium, *Leuconostoc citrovorum*, and in mice, an attempt was made to prevent the anti-leukemic effect of 4 amino N^{10} methyl PGA by CF.

Mice inoculated with leukemia AK4 and treated with 4 amino N^{10} methyl PGA 2 mg/kg 3 times weekly for ten doses survived 2–3 times as long as the untreated controls. When 300,000 units of CF were injected prior to each injection of 4 amino N^{10} methyl PGA, the mice survived only slightly longer than the controls. At death these mice showed the enlarged spleen, liver, and nodes, with massive microscopic leukemic infiltration, characteristic of this strain of leukemia. The mice treated with 4 amino N^{10} methyl PGA, when sacrificed on the same day of the disease, showed no enlargement of viscera and little or no leukemic infiltration microscopically.

Amounts of free PGA corresponding to the small quantities of folic acid activity of the CF concentrate, as determined by microbiological assay with *Strep. fecalis*, have been previously demonstrated to be insufficient to prevent the anti-leukemic effect of 4 amino N^{10} PGA in this strain of leukemia.

THE EFFECT OF 2,4,6-TRIETHYLENEIMINO-*s*-TRIAZINE AND RELATED COMPOUNDS ON TRANSPLANTED MOUSE LEUKEMIA.

J. H. Burchenal, S. F. Johnston (by invitation), C. Chester Stock, M. L. Crossley (by invitation), and C. P. Rhoads. (Leukemia Section of the Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City, and the Calco Chemical Division of the American Cyanamid Company, Bond Brook, N.J.)

Because of their highly-reactive chemical nature, a group of ethyleneimine derivatives were screened for their chemotherapeutic activity against transplanted mouse leukemia. 2,4,6-Triethyleneimino-*s*-triazine and 2,4-diethyleneimino-*s*-triazine were found to cause a definite prolongation of survival time, but 35 other triazines not containing ethyleneimine groups were inactive. Tolylene diethyleneurea and hexamethylene diethyleneurea, both of which contain two ethyleneimine moieties but no triazine component, possessed chemotherapeutic activity. Further testing of 2,4,6-triethyleneimino-*s*-triazine demonstrated that it prevented the development of leukocytosis and retarded the leukemic infiltration of liver, spleen, and kidneys in transplanted leukemia when dosage was started 2 days after inoculation of the leukemia. When the compound was administered on the eighth day of the disease, a severe leukopenia occurred rapidly.

At 2–4 times the acute LD 50 dose, 2,4,6-triethyleneimino-*s*-triazine inactivated leukemic cells in the body of the donor mouse within 2 hours, so that successful transplantation was not possible.

All these data make it appear that 2,4,6-triethyleneimino-*s*-triazine exerts its effect by virtue of the possession of at least two ethyleneimine rings and as such acts much like the nitrogen mustard group of compounds.

THE EFFECTS OF CORTISONE AND ACTH ON TRANSPLANTED MOUSE LEUKEMIA. Joseph H. Burchenal, C. Chester Stock, and C. P. Rhoads. (Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

Cortisone and ACTH have been assayed for their chemotherapeutic activity against transplanted mouse leukemia. With strain AK4 no definite prolongation of survival time was noted at daily doses varying from 30 to 100 mg/kg of cortisone or from 100 to 150 mg/kg of ACTH. The total daily dose was divided into from two to eight subcutaneous injections.

When 12.5 mg/kg of cortisone was given 8 times a day until the death of the mice on the sixth to ninth day, sections of liver showed slight to no leukemic infiltration, whereas heavy infiltration was noted in the mice given ACTH at a similar dose and dying at approximately the same time. Studies are under way at the present time attempting to protect against the toxic while preserving the antileukemic effects of high doses of cortisone.

In the mice treated with cortisone starting 48 hours after the inoculation of the leukemia, total leukocyte counts were much lower than those of the control animals, and there was a definite decrease in thymic weights of the treated mice. No significant difference was noted in adrenal weights of the two groups. In mice with far advanced AK4 leukemia, untreated for 8 days after inoculation, the mean leukocyte count fell from 114,000 to 36,000, 24 hours after a single subcutaneous injection of 250 mg/kg of cortisone. In similar doses ACTH was somewhat less active by these technics.

STUDIES ON *DROSOPHILA* TUMORS. Walter J. Burdette. (Department of Surgery, School of Medicine, Louisiana State University, New Orleans, La.)

Nine different stocks of *Drosophila* which develop spontaneous, melanotic tumors have been studied. Tumors have appeared sporadically in other cultures in the laboratory and probably are not uncommon elsewhere. The tumors, composed of polygonal and sometimes fusiform cells which appear in early larval life, later become pigmented and regress, leaving a residue of melanin. The presence of these tumors is determined by observing this residual pigmentation.

The incidence, size, and location of tumors vary widely when stocks are compared. For example, the incidence is 1 per cent in *tu 36e* flies and 50 per cent in the tu^g stock. In the tu^{wps} strain they are small and located about the head, and in the tu^g strain they appear as large abdominal masses. Changes in population size, nutrition, and temperature also alter the percentage of tumorous individuals. The number and location of genes for tumor susceptibility are unique for each strain. The main gene(s) responsible for tu^g has been traced to the second chromosome by means of appropriate crosses.

These tumor stocks are ideally suited for studies on the relationship between carcinogenic and mutagenic properties of chemical and physical agents. Control studies revealed a lethal mutation rate of 0.071 per cent in untreated females and 0.099 per cent in flies treated with 20-methylcholanthrene as an aerosol. However, it was 0.874 per cent in those treated with methyl-bis(β-chloroethyl)-amine hydrochloride. The numbers of X chromosomes tested were 2,822, 10,108, and 17,052 respectively. The *tu 36a* strain has been used to compare effects of these chemicals on tumor incidence and mutation rate simultaneously.

FURTHER STUDIES ON A POLAROGRAPHICALLY REDUCIBLE SUBSTANCE, THE NATURE OF WHICH CHANGES DURING CARCINOGENESIS. C. Carruthers and V. Suntzeff. (Division of Cancer Research, Dept. of Anatomy, Washington University School of Medicine, St. Louis, Mo.)

Recent observations have demonstrated that a substance extractable by lipid solvents from epidermis during carcinogenesis undergoes an alteration as detected by a difference in the type of polarographic waves and in their half-wave potentials. The reducible substance from epidermis and the carcinoma was present in the acetone-soluble fraction of the lipids, and was further purified by counter-current distribution and by the removal of alcohol-soluble fatty acids as the calcium salts. The substance from these tissues contains phosphorus, but no halides, nitrogen, or sulphur, and it is extremely hygroscopic and not soluble in nonpolar solvents. Its stability in 0.5 N HCl or 0.5 N NaOH at 100° C. for 1 hour was shown by little change in diffusion current or behavior in the ultraviolet. The substance from normal and hyperplastic epidermis absorbed maximally at 280 mμ in alcohol, and the maximum was pH dependent, while the substance from the carcinoma absorbed at 260 mμ and was pH dependent.

Experiments have shown that a transplantable rhabdomyosarcoma of mice contains a substance similar to that of the carcinoma with respect to polarographic behavior and absorption characteristics, but the substance from normal muscle is re-

ducible but does not absorb in the region of 260 mμ. No difference has been found to date between the substance from normal mouse liver and a transplantable hepatoma.

THE ISOLATION OF VISIBLE PROTOPLASMIC STRUCTURES. ROBERT CHAMBERS. (New York University, Washington Square College of Arts and Sciences, New York, N.Y.)

Investigators are increasingly attempting to isolate cell nuclei, mitochondria, and other visible protoplasmic structures from crushed masses of cells. One must be careful in evaluating the results of such experiments.

Puncture of a living cell with a microneedle or mechanically crushing a mass of living cells, whether in buffered or unbuffered media, always results in the development of an acid injury at a pH incompatible with protoplasm. There are at least two types of normally visible cell-inclusions: those which may be regarded as incidental or accessory to the vital properties of the protoplasm, e.g., yolk granules, pigment granules, etc.; and those vitally concerned with the properties of protoplasm. The former may not be seriously affected by tearing or crushing the cells, while the latter are. In a crushed mass of cells, in addition to the relatively unaffected accessory granules, the suspension consists of altered "nuclei," altered "mitochondria," and Lewis' so-called "death granules" which appear *de novo*. These considerations must be taken into account in any attempt at identifying isolated structures with those seen in living cells.

Mechanically destroying a cell produces various kinds of reactions which profoundly alter both chemical and spatial interrelationships. We are a long way from elaborating a medium which will permit the unaltered isolation of visible structures basic to the life of the cell.

CYTOLOGIC ALTERATIONS IN PULMONARY TUBERCULOSIS WHICH SIMULATE CARCINOMA. H. DAVIS CHIPPS and LOUIS H. KRAUL (by invitation). (Department of Pathology, School of Medicine, University of Washington, Seattle, Wash.)

Many investigators have shown that cytologic examination of exfoliated cells from the bronchus may be a very useful aid in the diagnosis of bronchogenic carcinoma. The cytologic stigmata of exfoliated cancer cells have been described and beautifully illustrated in the literature.

It is the purpose of this study to emphasize that atypical epithelial cells can be found in smears made from bronchoscopic washings and sputa from patients that do not have carcinoma. Tuberculosis is the most important noncancerous chronic disease of the bronchial tree, and in this study from one to nine specimens collected at different times from 240 patients with proved pulmonary tuberculosis were examined. The specimens were studied by the Papanicolaou smear and stain method as well as by making conventional sections from paraffin blocks. Nine (3.7 per cent) of the 240 cases had bronchogenic carcinoma co-existent with pulmonary tuberculosis. The other patients, 231, have been followed from 1 to 3 years, and, in so far as can be determined, they do not have carcinoma. The bronchogenic washings or sputa from this latter group contained many atypical cells that simulate cancer cells and which must be differentiated from cancer cells. These atypical noncancerous cells will be illustrated by lantern slides of colored photomicrographs.

GROWTH OF SARCOMA 37 BEFORE AND AFTER VASCULARIZATION. C. H. U. CHU (introduced by John J. Biesele). (Sloan-Kettering Institute for Cancer Research, New York 21, N.Y.)

Intramuscular Sarcoma 37 from the hind leg of a CAF mouse was transplanted subcutaneously into CAF mice. As controls, muscular tissue from the other leg was transplanted similarly. Serial sections were made from specimens taken every 2 hours for 24 hours and every day for 12 days.

Muscle implants were found to degenerate by loss of cross-striation and nuclei. Macrophages penetrated into the degenerating fibers, and giant cells surrounded the fiber to form multinucleated giant cells. The implant finally disappeared, and the subcutaneous tissue returned to normal.

Vascularization of Sarcoma 37 occurred 3–4 days after inoculation. Prior to vascularization, there was a rearrangement of cells into three zones: (*a*) the peripheral zone of giant cells, (*b*) the intermediate zone of spindle cells, and (*c*) the central zone of necrotic cells. If vascularization failed, as in a "dormant tumor," the central necrotic zone increased in amount, the intermediate zone disappeared, and the peripheral zone was reduced to a few giant cells. The tumor remained small.

Granulation tissue around the implant brought in more macrophages and giant cells. After the incorporation of the granulation tissue into the implant, a new layer of granulation tissue formed around the growing tumor. Well-vascularized tumors contained a mixture of spindle and necrotic cells. Vascularization seemed to supply not only

nutriment but also cellular elements for the growth of the daughter-tumor.

EYE TUMORS IN FEMALE RATS PRODUCED BY FEEDING 2-ACETYLAMINOFLUORENE. D. H. Copeland and R. W. Engel. (Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala.)

Eye tumors appeared in weanling female rats when they were fed semisynthetic diets low in fat and containing 0.03 per cent 2-acetylaminofluorene. This type of tumor appeared in 200–300 days in 6 of 19 animals (32 per cent incidence) receiving the fat-low diets. None of these animals developed mammary tumors. Control animals receiving the same diets but modified to contain 15–20 per cent lard, at the expense of sucrose, developed mammary tumors in approximately 140 days, a confirmation of previous experiences (*Cancer Research*, 9:608, 1949).

Although tumors are produced in a variety of sites by this carcinogen, as reported by other investigators, tumors of the eye have not been reported previously. The histologic characteristics of this new type of 2-acetylaminofluorene-induced tumor will be illustrated.

THE DESOXYRIBONUCLEIC ACID CONTENT PER NUCLEUS IN NORMAL, PRECANCEROUS, AND CANCEROUS TISSUES OF THE RAT. Lew Cunningham (by invitation), A. Clark Griffin, and J. Murray Luck. (Department of Chemistry, Stanford University, Calif.)

Other investigators have established that the desoxyribonucleic acid content of individual nuclei of various types of somatic cells is constant for animals of any one species and very similar for different species of mammals. Increased whole tissue content of desoxyribonucleic acid is characteristic of many tumors and of certain organs during carcinogenesis. Therefore, nuclei were isolated, by a modification of Dounce's method, from normal rat liver, from tumors produced in rats by feeding acetylaminofluorene, and from livers of rats which showed precancerous changes caused by feeding acetylaminofluorene or the carcinogenic azo dye, 3′-methyl-4-dimethylaminoazobenzene. The nuclei were counted on hemocytometer slides, and desoxyribonucleic acid was determined on suspensions containing known numbers of nuclei. A practically constant value, closely approximating 6×10^{-6} μg. of desoxyribonucleic acid per nucleus, was found for these tissues, in good agreement with reported values for other mammalian somatic cells. Evidence of polyploidy was occasionally encountered. The study is being extended to other types of tumors.

EFFECTS OF HORMONES ON CONCENTRATION OF VITAMIN A IN THE BLOOD OF CANCER PATIENTS. Abram Danish and Calvin T. Klopp (introduced by Paul K. Smith). (Departments of Medicine and Surgery, George Washington University School of Medicine, Washington 5, D.C.)

It has been demonstrated that the concentration of vitamin A in the blood stream of patients with gastro-intestinal and other forms of cancer is below normal in a majority of patients. This abnormality cannot be corrected by vitamin A therapy. A study of the mechanism involved was undertaken. This involved an evaluation of the possible role which the sex hormones might play. The effect of the administration of testosterone and estrogens was studied in both cancer and non-cancer patients. A consistently reproducible effect was noted—namely, an increase in concentration of the vitamin A in the blood following administration of testosterone or stilbestrol. The response in relation to testosterone is affected by the solvent used and up to a certain level is proportional to the dose. This interrelationship between hormone and vitamin is being further defined, and additional hormones are being studied. The results available at this time are too fragmentary to permit conclusions as to the mechanisms involved, but experiments have been set up for this purpose.

FURTHER STUDIES ON NATURAL ANTI-SHEEP AGGLUTININS IN MICE OF INBRED STRAINS. Israel Davidsohn and Kurt Stern. (Chicago Medical School and Mount Sinai Medical Research Foundation, Chicago, Ill.)

Previous observations on the common occurrence of natural antisheep agglutinins in C57 black mice, in contrast to other inbred strains, were confirmed on a larger material. Attempts were made to throw light on this difference. Analysis of 220 C57 blacks (3–20 months old) showed disproportionately high incidence of animals with absent or low-titered agglutinins in litters linked by common ancestry. This suggests the presence of genetic determinants in the occurrence of the antibodies. Preliminary results of hybridization experiments (C57 black X C3H, C57 black X dba) seem to support this assumption.

The role of age was studied by determining agglutinins in eight age groups (5–12 weeks) of C57 blacks, each group consisting of 30–40 animals.

The percentage of animals with antisheep agglutinins was lowest in the youngest group (5 weeks) and rose gradually up to the age of 12 weeks, indicating the postnatal origin of the antibodies.

The possibility was considered that there is an inverse relationship between the presence of antisheep agglutinins in the serum and the presence of corresponding antigens in tissues of the animals. However, natural antisheep agglutinins of C57 black serum were not absorbed by erythrocytes, liver, spleen, and kidney suspensions of C57 black, C3H or dba animals. Furthermore, injections into rabbits of erythrocytes, liver, spleen, and kidney of the three strains showed no relationship between the strain origin of the antigens and the production of antisheep agglutinins and hemolysins. Likewise, absorption experiments showed no difference in the behavior of tissues derived from different strains. Antiserums against sheep erythrocytes produced in rabbits and guinea-pigs, treated with tissues of the three strains, failed to reveal antigenic differences of Forssman type in the examined tissues.

MILK AGENT AND NATURAL ANTISHEEP AGGLUTININS IN MICE OF INBRED STRAINS. Israel Davidsohn, Kurt Stern, and John J. Bittner. (Division of Cancer Biology, Department of Physiology, University of Minnesota Medical School, Minneapolis 14, Minn.; Chicago Medical School and Mount Sinai Medical Research Foundation, Chicago, Ill.)

Natural antisheep agglutinins have been found to occur in more than 90 per cent of C57 black mice but in a much lower percentage of mice of five other strains (Davidsohn and Stern, 1949). Since three strains with low incidence of agglutinins (C3H, dba, Marsh-albino) carry the milk agent and since C57 black mice with high incidence of agglutinins do not carry the agent, the possible relationship between presence of the milk agent and of the natural antisheep agglutinins was investigated. In three strains—Z (C3H), A, D_2—animals with and animals without the milk agent were tested for agglutinins. The incidence of agglutinins was generally low (less than 30 per cent) but was not influenced by presence or absence of the milk agent. In addition, a low incidence of agglutinins was also found in mice of strain I, which does not carry the agent.

STUDIES ON METASTASIS; THE EFFECT OF ANATOMICAL LOCATION ON THE GROWTH OF TUMOR IMPLANTS. Robert P. DeLong (by invitation) and Dale Rex Coman. (Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia 4, Pa.)

In a previous paper, evidence was presented that the location of metastases depends primarily on the anatomical distribution of the vascular system and the availability of tumor emboli, rather than upon favorable or unfavorable local conditions, the "soil," at the site of arrest of the embolus. In the present experiments the effect of local conditions on neoplastic growth was tested by implanting fragments of tumor into several organs and measuring the size of the resulting tumors. Tumor implants were made in the adrenal, kidney, liver, spleen, and muscle of rabbits, rats, and mice. V_2 carcinoma and Brown-Pearce tumor mice were used in rabbits, Walker rat carcinoma 256 in Long-Evans hooded rats, and carcinoma 241 in C57 mice. About 2 weeks later the animals were sacrificed, and the tumors were removed and weighed. In C57 mice, in rabbits bearing the Brown-Pearce tumor, and in rats with Walker 256 carcinoma, no significant differences in size were found among the tumors in the different organs. In rabbits bearing V_2 carcinoma, the tumors in the spleen were significantly larger than those in other organs. It is concluded that under these conditions, the rate of neoplastic growth is generally the same in the several organs tested, differences being exceptional. This result supports our earlier conclusions that the susceptibility of organs to the establishment and growth of secondary tumors, the "soil," plays only a minor part in the distribution of metastatic tumors.

FUNGI ASSOCIATED WITH TUMOR TISSUES. Irene Corey Diller. (Institute for Cancer Research, Philadelphia, Pa.)

The microscopic study of a variety of tumors by means of cytological technics not ordinarily applied to tumor tissue has led to the observation that there are present in these tissues spores and other fungal elements. These organisms are readily isolated from tumor tissue on Sabouraud's agar. They could not be isolated from the normal tissues of mice bearing transplanted tumors but were found in the mammary tissue of C3H female mice that had not yet developed tumors. Mycelial forms were isolated from transplanted, chemically-induced, and spontaneous tumors of the mouse and from many types of human tumors, and a yeast-like form was obtained from all the blood-forming tissues of leukemic mice. Preliminary indications are that the fungi (usually Phycomycetes or Imperfecti) are not ordinary laboratory contaminants, and that a specific fungus (or specific fungi)

are associated with each different type of tumor tested. Immunological experiments are being conducted in the hope of elucidating the host-parasite relationships.

ADRENAL FUNCTION IN PATIENTS WITH NEOPLASTIC DISEASE. KONRAD DOBRINER, SEYMOUR LIEBERMAN (by invitation), HILDEGARD WILSON (by invitation), and C. P. RHOADS. (Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

We have reported that in patients with neoplastic disease, the excretion of adrenal cortical and gonadal steroid metabolites is markedly diminished. Often, certain steroids are no longer demonstrable, and a compound of adrenal cortical origin, 11-hydroxy-etiocholanolone, is found in the urine of a significant number of patients. The alteration in steroid hormone production in patients with neoplastic growth as evidenced by the steroid excretion patterns has been investigated after the administration of ACTH and Cortisone acetate. There is great individual variation to the prolonged administration of ACTH measured by the excretion of steroids; while many patients respond readily to 100 mg/day of the hormone, several instances have been observed where an effect was found only after 200 mg/day had been given. This indicates that the adrenal gland was relatively unresponsive in these subjects.

The excretion patterns of the individual steroids reveal an increased excretion of metabolites of the C-19 and C-21 series with and without 11-oxygen function. A variety of unidentified compounds not observed in the urine of normal or diseased persons were found. Certain other steroids, previously detected only in Cushing's syndrome, were excreted. The variations in the steroid patterns of individual patients reflect differences in adrenal hormone production. It is also possible that an alteration in metabolism occurs so that new end products and intermediates are excreted in the urine.

The clinical changes which have been observed in patients with neoplastic growth during administration of ACTH and Cortisone and their relationship to adrenal function and steroid metabolism will be discussed.

STUDY OF THE IODOACETATE INHIBITION OF THERMOCOAGULATION OF SERUM PROTEIN IN CANCER AND TUBERCULOSIS. GREGORY S. DUBOFF. (Research Laboratory, Emery Tumor Group, Los Angeles, Calif.)

Correlation of the two factors, serum total protein (6–8 per cent normal for procedure) and smallest amount of acetate required to block development of firm, opaque clot on thermocoagulation (modification submitted privately by Dr. Huggins), and relating these to the various clinical states tested was found to be:

Subjects	No. of cases	Iodoacetate index	Total protein gm/ 100 ml. serum (Per cent)
Early cancer	24	4.7– 8.8	6.1–7.7
" "	22	9.0–13.2	5.9–6.8
Advanced cancer considered uncontrolled	23	6.2– 8.1	4.4–5.7
Advanced cancer considered controlled	13	9.5–12.2	6.6–7.5
Active pulmonary tuberculosis	44	5.3– 7.3	4.3–5.1
Arrested pulmonary tuberculosis	11	9.3–11.4	6.3–7.2
Benign neoplasia	78	9.2–16.0	5.9–7.6
" "	14	5.4– 8.4	5.8–8.1
Non-symptomatic normals:	83	9.3–11.3	6.2–8.2
" "	2	7.7– 8.9	6.5–6.7

It is apparent that in the presence of a moderate to marked hypoproteinemia (6 per cent or less) as demonstrated in the serum of the tuberculous and advanced cancer cases tested in this investigation, a positive iodoacetate index (less than 9) is in no way related to the positive iodoacetate index obtained for the nontreated early cancer cases, whose sera yielded a total protein within the normal range, although the index range falls well within that of the two former clinical states.

THE EFFECT OF ADDED DIETARY TRYPTOPHANE ON THE OCCURRENCE OF INDUCED CANCER IN RATS. W. F. DUNNING, M. R. CURTIS, and M. E. MAUN. (Detroit Institute of Cancer Research and Department of Pathology, Wayne University College of Medicine and Deaconess Hospital, Detroit, Mich.)

Three groups of A×C line 9935 and four groups of Fischer line 344 female rats were placed on similar isocaloric synthetic diets containing 26 per cent protein. The A×C rats had diethylstilbestrol pellets implanted in their scapular region, and the Fischers received 0.06 per cent acetylaminofluorene in their diet. One group of each line received the diet containing 26 per cent casein, another the same diet, substituting 24.6 per cent tryptophane-free casein and 1.4 per cent DL-tryptophane for the intact casein, and a third group received in place of the intact casein 21.7 per cent tryptophane-free casein plus 4.3 per cent DL-tryptophane. The fourth group of Fischers received 45 per cent casein.

Of the A×C rats, 39 survived the minimum latent period, and 32 developed 161 gross and a total of 436 mammary cancers, while 51 Fischers

survived the minimum latent period and 34 developed liver cancers, 22 developed bladder cancers, and 5 developed cancers of the external auditory meatus.

The addition of 1 per cent DL-tryptophane to the diet increased the number and percentage of induced mammary cancers and significantly reduced the average latent period below that observed for the control group, while the addition of 4 per cent dietary tryptophane reduced the number and percentage of induced mammary cancers and significantly prolonged the latent period. Added dietary tryptophane did not affect the incidence of acetylaminofluorene-induced liver cancer, but bladder cancers were confined to rats on high-tryptophane diets and occurred in nearly 100 per cent of them.

FURTHER STUDY ON THE RECOVERY OF 2-ACETYLAMINOFLUORENE FROM RATS FOLLOWING ORAL ADMINISTRATION. HELEN M. DYER, HELEN E. ROSS (by invitation), and HAROLD P. MORRIS. (National Cancer Institute, Bethesda, Md.)

To explain the rapid disappearance of acetone-extractable acetylaminofluorene (AAF) from tissues of rats after oral administration, it was suggested (Morris and Westfall, *J. Nat. Cancer Inst.*, **9**:149, 1948) that AAF may be rapidly inactivated or that it may enter into chemical combination with some body constituent and that the combination might be insoluble in the acetone which was used for extraction.

The second possibility has been subjected to preliminary test. AAF was administered in single large doses or was fed continuously in the diet for periods of 2–3 weeks. Using a slight modification of Westfall's diazotization method (*J. Nat. Cancer Inst.*, **8**:17, 1947), ether extracts of saponified lipids of the tissues were analyzed, in addition to unsaponified acetone extracts. Acetate-buffer-soluble protein fractions and acetone-extracted residues of tissues were hydrolyzed by trypsin and by alkali, and the hydrolysates were extracted with ether. The ether residues were analyzed.

The observations of Westfall and Morris on acetone extracts were confirmed. Moreover, no AAF, AF, or HCl-hydrolyzable combinations were found liberated by saponification of lipids or by hydrolysis of protein fractions.

It is concluded that AAF does not combine in the tissues of the rat with acetate-buffer-soluble proteins or with saponifiable lipids—at least, in such combinations as may yield diazotizable AAF upon hydrolysis.

RESULTS OF THE INJECTION OF CULTURED FIBROBLASTS INTO STRAIN C3H MICE. W. R. EARLE, E. SHELTON (by invitation), and E. L. SCHILLING (by invitation). (National Cancer Institute, Bethesda 14, Md.)

Six strains of cultured fibroblasts, of which five had been treated with 20-methylcholanthrene, were tested for ability to give rise to tumors *in vivo* by injecting aliquots into C3H mice. The strains were grown for 4 years after treatment; tumor-producing capacity was tested 5 times during this period. Curves plotted for these five series of injections show the relationship of percentage of tumors produced to the time during which cells had been treated with carcinogen. While the shape of all curves is the same, the level for each falls with time, reflecting reduction of ability to produce tumors with time.

Comparison of the shape of the curves with the progressive changes in the morphology of the cell strains indicates that the increasing degree of morphologic alteration sustained by these strains when treated with the carcinogen cannot be correlated directly with their ability to produce tumors when injected into mice. Rather, it appears that the cells may become so greatly altered that they are less able to adjust themselves to life *in vivo* and so produce fewer tumors than the less altered cells.

The gradual lowering of the level of the whole curve with the passage of time suggests that culturing cells over extended periods of time in completely heterologous media may alter the reaction of the cells to their specific host, so that the cells are less able to survive when returned to the host environment.

THE SIGNIFICANCE OF THE ANTERIOR CHAMBER IN TUMOR TRANSPLANTATION ACROSS STRAIN BARRIERS. E. J. EICHWALD (introduced by T. F. Dougherty). (Dept. of Pathology and Oncology, University of Utah Medical School, Salt Lake City, Utah.)

Greene's statement that "the so-called influence of strain barriers is largely negated when the anterior chamber is used" in transplantation of tumors has been subjected to a review.

Neoplasms were transplanted into the anterior chamber to determine whether the strain barrier would limit growth in this area in the same way that tumors are confined by a species barrier. If the tumors of the same strain were not limited, their subsequent course would be one of regression or of progressive growth. It was kept in mind that

short-lived transient growth might occur as well subcutaneously as in the anterior chamber but escape detection because of the overlying coat.

The method of serial sacrifice was chosen. The tumor selected was neuroblastoma C1300 and the mouse strain C57 brown.

Tumor growth in the anterior chamber was observed in most animals, while only a small number of the subcutaneously inoculated animals showed growth. In almost all animals, the growth started in the anterior chamber, extended beyond it, and deeply invaded the periocular structures. Many of these tumors regressed after 3–5 weeks. In others, growth continued progressively until death. This was significantly more common than in the subcutaneously inoculated animals. It is concluded that, by selecting the anterior chamber (of this strain) as a transplant site (for this tumor), both progressive or transient growth can be obtained in animals which would not have proved susceptible if inoculated subcutaneously.

DIETARY FACTORS INFLUENCING THE CARCINOGENICITY OF 2-ACETYLAMINOFLUORENE.

R. W. Engel. (Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala.)

Voluntary caloric restriction and consequent slow body weight gain resulted in a low incidence (25 per cent) of mammary tumors and a high incidence (75 per cent or higher) of face and liver tumors in weanling female rats of the AES strain. When food intake approached more normal levels and weight gains of 120 gm. or more were attained in 16 weeks, mammary tumors consistently developed (98 per cent incidence) in an average of 20 weeks. The diet used was semi-synthetic and contained 11 per cent protein, 15 per cent fat, and 0.03 per cent 2-acetylaminofluorene.

Increasing the dietary protein to 20 or 27 per cent (casein), at the expense of sucrose, resulted in more rapid weight gains but failed to influence tumor incidence or induction time. However, when the dietary protein was increased to 40 or 60 per cent the tumor incidence was decreased to 25 per cent or less, and survival time was extended considerably (4–16 weeks).

Replacing the fat in the diet with an equal amount of sucrose resulted in a low incidence of mammary tumors (10 per cent) and a relatively high incidence of eye tumors (32 per cent). Weight gains averaged 60 gm. in 16 weeks among the animals receiving the fat-low diets.

Animals receiving a complete diet (stock diet for rat breeding colony) containing 0.03 per cent 2-acetylaminofluorene remained tumor-free for 40 weeks.

STUDIES OF VARIOUS TESTS FOR MALIGNANT NEOPLASTIC DISEASES. I. THE REDUCTION OF METHYLENE BLUE BY PLASMA.

Nils Eriksen (by invitation), Lester D. Ellerbrook (by invitation), and Stuart W. Lippincott. (National Cancer Institute, Bethesda 14, Md., and the Department of Pathology, School of Medicine, University of Washington, Seattle, Wash.)

The methylene blue reduction technic developed by Black has been applied to specimens of plasma obtained from fasting normal individuals and fasting hospital patients with various neoplastic and non-neoplastic diseases. The control group consisted of 120 normal individuals, ranging from 18 to 66 years of age, all of whom at the time of testing had normal temperatures, normal white blood cell counts, and chest x-rays indicating no active tuberculosis, and were considered in good health; 109 patients with neoplastic diseases, proved by microscropic section, were tested prior to any form of treatment—in this group were 92 cases of malignant neoplasms and 17 cases of benign neoplasms. In addition, 21 patients with malignant neoplasms were tested following operation. The group of miscellaneous non-neoplastic diseases was represented by 130 cases.

A reduction time exceeding the lowest 95 per cent of the normal reduction times was arbitrarily considered a positive result. For the various groups described, the following percentages of positive results were obtained: pre-treatment malignant neoplasms, 53 per cent; post-treatment malignant neoplasms, 52 per cent; benign neoplasms, 24 per cent; miscellaneous non-neoplastic diseases, 25 per cent.

The lack of sensitivity and specificity indicated by these results would seem to preclude use of the test for detection of cancer by mass-screening of a general population. The mechanism of the reactions involved in the test is not clearly understood. Moreover, certain technical aspects of the test complicate interpretation of the result obtained for a single specimen.

THE EFFECT OF TESTOSTERONE ON THE PITUITARY–ADRENAL CORTEX MECHANISM IN PATIENTS WITH BREAST CANCER.

William W. Faloon (by invitation), Lawrence A. Owens (by invitation), Margaret C. Broughton (by invitation), and L. Whittington Gorham. (Division of Oncology and the Department of

Medicine, Albany Medical College, Albany, N.Y.)

In normal individuals, destruction of 50 per cent or more of circulating eosinophils occurs following either ACTH (adrenocorticotropic hormone) injection or epinephrine stimulation of pituitary ACTH secretion. Epinephrine thus may be used as a test of integrity of the pituitary ACTH, adrenal 11, 17-oxysteroids, eosinophil destruction sequence. (Pituitary Adrenal Cortex Mechanism or PACM. Recant, L.; Forsham, P. H.; and Thorn, G. W. Observation on the Pituitary-Adrenal Response following Epinephrine Infusion in Man. *J. Clin. Endocrinol.* **8**:589, 1948.)

This test has been applied to the study of six women with breast carcinoma, one with uterine carcinoma, and three with noncancerous conditions. During continued administration of testosterone propionate, 12.5–100 mg. daily, 0.3 mg. of epinephrine hydrochloride was given at 2–10 day intervals and eosinophil counts done.

Of six breast carcinoma cases, receiving described androgen therapy, three showed consistent normal falls in eosinophils and no clinical improvement. Three others showed subnormal falls and clinical improvement. Three of the four nonbreast cancer cases also showed subnormal falls whereas two normal women (controls) receiving no testosterone gave repeatedly normal eosinophil decreases after epinephrine.

In one patient (noncancer), who developed subnormal responses, cessation of androgen was followed by return of normal epinephrine response. In two patients (one cancerous), reacting subnormally to epinephrine during androgen therapy, ACTH injection yielded normal eosinophil destruction.

Foregoing observations suggest inhibition of PACM produced by testosterone. This inhibition appears caused by reduced pituitary ACTH secretion following epinephrine stimulation.

In a small series of cases of advanced breast cancer, diminished pituitary function apparently parallels clinical improvement. This finding may not be of causal significance, but merely reflection of other decreased pituitary function.

ULTRAVIOLET ABSORPTION STUDIES OF INTERSTITIAL CELL LIPOID OF THE TESTIS IN NORMAL AND IN STILBESTROL TREATED MICE. Harlan I. Firminger (introduced by Harold L. Stewart). (Pathology Section, National Cancer Institute, Bethesda 14, Md.)

Interstitial cells of the testis have long been thought to be the source of testosterone in males. Histochemical technics have indicated the presence of large amounts of lipoid with an associated carbonyl group, but the final proof has not yet been secured as to the true chemical nature of this lipoid. The results of a preliminary study using ultraviolet absorption spectromicroscopy of the testicular interstitial cells of normal and of stilbestrol treated mice will be presented and discussed. It is hoped this technic will provide more specific information as to the chemical character of this product of the interstitial cells.

FACTORS IN THE EVALUATION OF β-GLUCURONIDASE ACTIVITY IN CANCER OF THE UTERINE CERVIX. William H. Fishman, S. Charles Kasdon (by invitation) and F. Homburger (Cancer Research and Cancer Control Unit, Departments of Surgery, Medicine, and Biochemistry, Tufts College Medical School, Boston, Mass.)

Previously (*Cancer Research*, **7**:12, 1947; *Cancer Research*, **9**:609, 1949), β-glucuronidase activity of neoplastic tissues and exudates was investigated. Observations have now been made on the relationship of vaginal fluid activity and the incidence of cervical cancer. Vaginal secretions in 500 nonpregnant healthy women were assayed for β-glucuronidase activity, and values from 0 to 4,500 units per gram were encountered with the majority below 300 units, with some 34 per cent above this arbitrary level. In the older age groups, values tended to be greater than 300 units. Factors other than pregnancy, trichomonas, uterus and/or cervix have been implicated in the explanation of vaginal fluid glucuronidase activity.

Assays of tissue biopsies from cervical cancer consistently showed β-glucuronidase activity far above the levels found in nontumor tissue adjacent to the lesion and in the benign cervix. However, the vaginal fluid in seven out of twenty patients with untreated cancer of the cervix contained glucuronidase activity below 300 units per gram. Accordingly, the concept (Odell, L. D., and Burt, J. C., *Cancer Research*, **9**:363, 1949) that cancer of the cervix consistently provides an excessive supply of glucuronidase for the vaginal secretions is not supported by the present data. However, the elevation of glucuronidase activity in the malignant cervical tissue is uniformly found, and its significance in cancer warrants further investigation.

INFLUENCE OF "HORMONES" PLAYING ROLE IN NORMAL METAMORPHOSIS ON CHEMICAL CARCINOGENESIS. W. R. Franks, Roy Bather (by invitation),

and J. S. THOMPSON (by invitation). (Banting and Best Department of Medical Research, University of Toronto, with Grant-in-Aid from the National Cancer Institute of Canada.)

In all species, the incidence of spontaneous cancer is relatively low during the period of active normal tissue differentiation. A study has been instigated of the possible role of factors known to play a part in the normal development of carcinogenesis, using methylcholanthrene and dibenzanthracene experimentally in mice. Two preparations have been studied: (*a*) a pupating "hormone" isolated from *Calliphora* larvae by a modification of the method of Becker and Plagge, which plays a role in the normal pupation of insect larvae but was found to have no effect on metamorphosis of tadpoles, and (*b*) thyroxine, which is well known to hasten the metamorphosis of tadpoles (and mammals) but was found to have no effect on insects. The former contains 12 per cent bromine, the latter iodine. Both of these substances have shown an inhibitory effect on carcinogenesis, the former on the carcinogenesis of methylcholanthrene painting and both substances on dibenzanthracene injections in mice ($P < .01$).

A control group using bromobenzene (Crabtree) in equivalent bromine amounts to the pupating "hormone" has also been set out. A preparation of "prepupating" substance collected from the insect larvae at an earlier stage in their development by the same technic does not apparently show the same degree of activity in inhibiting carcinogenesis induced by dibenzanthracene.

INFLUENCE OF BACKGROUND RADIATION ON METHYLCHOLANTHRENE CARCINOGENESIS IN MICE.

W. R. FRANKS and G. A. MEEK (by invitation). (Banting and Best Department of Medical Research, University of Toronto, with Grant-in-Aid from National Cancer Institute of Canada.)

Eugster and Hess (1940) reported a fourfold reduction in the incidence of spontaneous mouse cancer by reducing to one-twentieth a background radiation of 8 ions/cc/second. Figge (1947) reported a significant increase in methylcholanthrene carcinogenesis by increased exposure to secondary radiation from cosmic showers. George and Horning (1948) were unable to show an increase in carcinogen tumor production by a fivefold increase in cosmic ray intensity at 12,000 feet. Results from part of our experiments carried out in co-operation with the McIntyre Research Foundation are now apparent.

Methylcholanthrene was injected into pure strain mice and these divided into three groups totaling 230 each and kept under the following conditions: (*a*) surface control group in Toronto with background radiation intensity equal to 6 ions/cc/second, (*b*) a "radiation free" laboratory half a mile underground in a mine, where the radiation background was not greater than 0.4 ions/cc/second, and (*c*) control underground laboratory on the same level as (*b*) but where the rock radiation was approximately equal to the Toronto background.

No significant difference in tumor incidence has been found between any of these groups, either in the over-all numbers or in the length of time to produce tumors. (Surface radiation, 112 tumors; underground control, 98; "radiation free" underground, 100.) Expressed as biological equivalents, no significant difference in methylcholanthrene carcinogenesis has been found by reducing to one-tenth, a radiation of approximately one ion-pair in 400 tissue-cells per hour.

STUDIES ON THE EFFECT OF FURACIN ON THE GROWTH OF A FIBROSARCOMA IN MICE.

CHARLES E. FRIEDGOOD and MORRIS N. GREEN (introduced by E. V. Cowdry). (From the Department of Surgery, Maimonides Hospital, Brooklyn, and the Department of Bacteriology, School of Medicine, University of Missouri, Columbia, Mo.)

Furacin (5-nitro-2-furaldehyde semicarbazone) is effective in delaying the growth of sarcoma S-13 in C3H mice of the Andervont strain. The controls lived on an average of 20 days while the furacin-treated animals survived on an average of 40 days, depending on the amount of furacin given. Furacin was injected subcutaneously in peanut oil at a region remote from the tumor. Sections of the treated tumors showed marked cellular degeneration with pyknotic nuclei along with a marked decrease in mitosis as distinguished from the untreated controls. Hypertrophy of the adrenal cortex with an enlargement of the fascicular zone was observed. Atrophy of the testis and ovaries was also observed when large doses of furacin were given over a longer period of time. In a series of controls, Wistar rats given large doses of furacin developed symptoms of multiple vitamin deficiency which disappeared upon discontinuance of the drug and when a diet fortified with vitamins was fed. No evidence of vitamin deficiency was observed in the C3H mice under the conditions of the tumor therapy experiments. Some possible theories of the mechanism of action of furacin on tumor growth will be discussed.

THE COMPARATIVE MORPHOGENESIS OF EXTRAGENITAL AND GONADAL TERATOID TUMORS.

NATHAN B. FRIEDMAN. (Armed Forces Institute of Pathology, Washington, D.C., and Division of Laboratories, Cedars of Lebanon Hospital, Los Angeles, Calif.)

Testicular germinomas (seminomas) are tumors of primordial germ cells. Germinomas ripen into tumors which form somatic (teratomas) and trophoblastic (chorioepitheliomas) tissue. The incidence of adult teratomas is higher and of teratocarcinomas, chorioepitheliomas and germinal (embryonal) carcinomas is lower in the ovary than in the testis. This suggests either that tissue development from the same genetic material is influenced by the endocrine environment or that male and female germ cells carry different genetic potentialities for differentiation.

Since germinomas, germinal carcinomas, teratocarcinomas, chorioepitheliomas, and teratomas are all found in both the pineal and thymus, the teratoid growths of these organs must be considered of germinal origin. This observation favors the view that the primordial germ cells do not originate from the somatic elements of the urogenital fold. This makes it impossible to consider germinomatous cells derived from spermatic elements, and the term seminoma should be eliminated.

Teratomas, chorioepitheliomas, germinal carcinomas, and teratocarcinomas, but no germinomas, have been observed among the sacrococcygeal teratoid growths. For the present, as unequivocal a germinal morphogenesis cannot be ascribed to the sacrococcygeal teratoid tumors as it can be to the other extragenital teratoid growths. Many, if not all, of the retroperitoneal teratoid tumors are actually metastases from primary genital tumors, and the old observation that such a primary growth may disappear completely is reemphasized.

DISTRIBUTION STUDIES WITH NITROGEN MUSTARDS LABELED WITH RADIOACTIVE IODINE.

ORRIE M. FRIEDMAN and ALEXANDER M. RUTENBURG (by invitation), and ARNOLD M. SELIGMAN. (Department of Surgery, Beth Israel Hospital, and Harvard Medical School, Boston, Mass., and Department of Chemistry, Harvard University, Cambridge, Mass.)

A comparison of the rate of disappearance from blood and tissues and appearance in the urine of radioactivity after intravenous injection of diethyl-β-iodo^{131}ethylamine ($I^{131}NM$) and NaI^{131} indicates that a small portion of $I^{131}NM$ had reached tissues and penetrated cells in uncyclized form. The highest concentration of radioactivity was noted in blood, lung, and lymph nodes, a distribution conforming to the locus of clinical activity of the mustards. Lower concentrations were noted in kidney, liver, and Sarcoma 37 growing in the subcutaneous space. In Wistar rats there was also evidence for penetration of a small fraction of $I^{131}NM$ into cells. The highest concentration of radioactivity was noted in thyroid and blood, lower concentrations in lung, kidney, Bagg lymphosarcoma, and spleen. In a patient with reticulum-cell sarcoma of the tonsil, a higher concentration of radioactivity was found in tumor, blood, and muscle after $I^{131}NM$ than after NaI^{131} injection. The rate of disappearance from blood, however, was the same.

The rate of disappearance of radioactivity from blood and tissues and its appearance in the urine of mice after injection of methyl-bis-(β-iodo^{131}-ethyl)-amine ($I^{131}NM$) was about the same as for NaI^{131}. Within the limit of this technic, evidence that uncyclized mustard reached the tissues intact and penetrated cells was provided only for lung. The highest concentration of radioactivity was noted in blood, lymph nodes, spleen, lung, and kidney. Lower concentrations of radioactivity were noted in intestine and in tumor (Sarcoma 37).

BIOPSY AND STEROID-EXCRETION STUDIES IN CONGENITAL ADRENAL CORTICAL HYPERPLASIA.

LYTT I. GARDNER, RONALD C. SNIFFEN, ANIELA S. ZYGMUNTOWICZ and NATHAN B. TALBOT (introduced by Charles W. Hooker). (Children's Medical Service and Department of Pathology and Bacteriology, Massachusetts General Hospital, and Departments of Pediatrics and Pathology, Harvard Medical School, Boston, Mass.)

Observations are presented on a 12-year-old boy with congenital adrenal hyperplasia who has virilism, the classical Addisonian defect in Na and K metabolism, and apparently normal carbohydrate metabolism.

Virilism has been noted from birth. Adrenal biopsy at age 3 years showed hyperplasia of the adrenal reticularis with no definite abnormality in the other zones. At age $7\frac{1}{2}$ years the testes underwent massive growth together with proliferation of large reticularis-like cells as shown by testicular biopsy. There was also sudden increase of urinary 17-ketosteroids to very high levels at this time (200 mg/24 hrs). These 17-KS were predominately of the alpha-alcoholic type, and when separated

into 40 fractions by microchromatography there was a grossly abnormal distribution pattern.

Methyl testosterone was given orally for 33 days (90 mg/24 hrs). There resulted a decrease in urinary 17-KS and 11-oxycorticosteroid excretion concomitant with marked changes in the histological appearance of the testes. The large, reticularis-like cells were no longer in the majority, but, instead, small, dense cells predominated. The large cells often appeared as islands in a sea of small cells that were closely applied to them. All the nuclei seemed denser and more deeply staining and in the small cells were often deeply basophilic, shrunken and without plasmosomes.

The abnormal urinary 17-KS chromatographic pattern suggests not only a quantitative but also a qualitative disturbance in 17-KS precursor production. The effects of methyl testosterone therapy support the hypothesis that this boy's adrenal hyperplasia is due to excessive pituitary stimulation.

EFFECT OF ESTRADIOL BENZOATE AND TESTOSTERONE PROPIONATE ON X-RAY INDUCED LEUKEMIA IN MICE.

W. U. Gardner. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Roentgen rays are known to be leukemogenic in mice. Also, several investigators have demonstrated a leukemogenic action of estrogens. Testosterone propionate has inhibited leukemia in estrogen-treated mice (*Cancer Research*, **4**:73). Estrogens reduce the death-rate of mice given large doses of x-rays (*Endocrinology*, **32**:161).

In the present experiment, mice of the BC stock were used. Over 300 mice from 29 to 92 days of age (usually 40–60 days) were divided into six groups of approximately 50 animals each. Litter-mates were placed in different groups; 380–285 roentgen units total body irradiation were given mice of each of the groups. A few days later, one group of males and one of females were started on the following weekly treatment schedules: $\frac{1}{20}$ ml. sesame oil, 1.25 mg. of testosterone propionate in $\frac{1}{20}$ ml. sesame oil; and 16.6 μg. of estradiol benzoate in $\frac{1}{20}$ cc. of sesame oil.

The incidence of leukemia was 13 per cent and 30 per cent for the males and females given sesame oil at an average age of 223 days and 283 days, respectively; 7 per cent and 17 per cent for the testosterone propionate-treated mice at 222 and 324 days; and 65 per cent and 57 per cent of the estradiol benzoate-treated mice at 281 and 276 days. Intact males and testosterone propionate-treated males and females had a much lower incidence of leukemia than did the females or estrogen-treated animals. A number of animals over 500 days of age are living.

Five ovarian tumors have appeared in the sesame oil and testosterone propionate-treated groups—none in the estrogen-treated group.

A STUDY OF THE EFFECT OF THE VIRUS OF EASTERN EQUINE ENCEPHALOMYELITIS ON NORMAL AND TUMOR CELLS IN TISSUE CULTURE AND BY ELECTRON MICROSCOPY.

George O. Gey and Frederick B. Bang (by invitation). (Departments of Surgery and Medicine, The Johns Hopkins University School of Medicine, Baltimore 5, Md.)

The destructive action of the virus of eastern equine encephalomyelitis on a tumor cell derivative of a normal rat fibroblast has been studied in tissue culture. In roller-tube cultures, the virus destroys the tumor cells within 1–2 days and attains a high titer in the supernatant fluid. Only slight growth of the virus and no cell destruction was detected with the same inoculum of virus on the normal autogenous rat cells maintained in the same medium.

Analysis of the phenomenon by placing diluted inocula of virus on 1-day explants of the tumor cells in special chambers shows the development of foci of destruction in the periphery of the culture. These foci, under the electron microscope, are shown to consist of destroyed and disintegrated cells, showing in particular collapsed vesicles (ballooned mitochondria?), scattered pairs, chains and single virus particles; and occasional clumps of virus. Similar destructive effects of this virus on chick embryo cells is observed.

PHYSIOLOGICAL AND CYTOLOGICAL CHARACTERISTICS OF ANALOGOUS MAMMARY TUMORS AND THEIR RELATION TO RADIOSENSITIVITY.

Anna Goldfeder. (From the Cancer Research Laboratory, Department of Hospitals, City of New York, and New York University, New York, N.Y.)

Mammary tumors which arose in inbred mice, C3H and dba, proved to differ in their rate of growth, metabolic activity, and radiosensitivity. The rate of growth as judged by the latent period (time between implantation and detection of tumor growth) was about 18 days for the C3H tumor and about 6 days for the dba tumor. The metabolic rate as determined by the O_2 uptake and aerobic glycolysis *in vitro* was as follows: For the C3H tu-

mor the Q_{O_2} averaged 3.5 and the $Q_{O_{2G}}$ 8.6. For the dba tumor the Q_{O_2} averaged 5.8, the $Q_{O_{2G}}$ 25.2.

C3H tumors treated with x-rays *in situ* required doses ranging from 8–12,000 r, while dba tumors of similar size required doses ranging from 16–24,000 r for their total destruction. Thus the rate of growth, metabolic activity, and radiosensitivity of the C3H and the dba tumors were in proportion about 1:3.

Cytological studies of the C3H and dba tumors, using various staining methods, revealed a relatively greater mitotic activity prevailing in the faster growing dba tumors, as compared with that of the slower growing C3H tumors. A greater degree of recovery, as evaluated by mitotic counts, occurred in the faster growing dba tumors as compared to those of the C3H tumors following the same medium doses of x-radiation.

Multinucleated giant cells, increase in fibrous tissue, and "pearl" formations were noted in C3H tumors following irradiation, but the same were not manifested in the dba tumors. The significance of these findings regarding a physiological classification of tumors and radiosensitivity will be emphasized.

REVERSAL OF THE ACTION OF A FOLIC ACID ANTAGONIST, 4-AMINOPTEROYLGLUTAMIC ACID, IN *DROSOPHILA MELANOGASTER*.

E. D. Goldsmith and Morris H. Harnly. (Department of Histology, College of Dentistry, and Washington Square College of Arts and Sciences, New York University, N.Y.)

Aminopterin, 4-aminopteroylglutamic acid, a PGA antagonist, was incorporated in Pearl's synthetic medium and was fed to the Woodbury Wild-type strain of fruit fly, *Drosophila melanogaster*. Levels of 1.0, 2.5, 5, 10, and 20 mg. per cent of the 4-amino PGA gave survival values of 58, 52, 22, 10, and 0 per cent, respectively, as compared with 79 per cent for untreated controls.

Addition of folic acid (PGA) at levels of 300–1,000 mg. per cent to the 5 mg. per cent aminopterin ration resulted in an emergence value of 70 per cent. Supplementation with 5 and 10 mg. per cent desoxyribonucleic acid (DNA) increased emergence to 55 and 65 per cent, respectively. Larger amounts of the DNA were not more effective. Thymine, when supplied at a concentration of 100 mg. per cent, resulted in a 52 per cent survival as compared to 42 per cent for the 50 and 150 mg. per cent levels. The yield on a 100 mg. per cent yeast nucleic acid plus 5 mg. per cent aminopterin ration was no different than that on 4-amino PGA alone.

Suitable combinations of DNA and PGA resulted in emergence values equal to those of the controls. Thymine-DNA combinations were no better than DNA alone.

The possible significance of these findings will be discussed.

THE EFFECT OF TWO LINKED COLOR GENES UPON THE ATYPICAL GROWTH OF ERYTHROPHORES AND MACROMELANOPHORES TO FORM ERYTHROMELANOMAS IN FOUR GENERATIONS OF HYBRID FISHES.

Myron Gordon and Ross F. Nigrelli. (New York Aquarium, New York Zoölogical Society, New York, N.Y.)

Fishes are particularly valuable for studies of normal and atypical pigment cell growth. Pathologists who have studied and compared the black pigment-cell tumors in the representatives of the six Classes of vertebrates discovered that the melanoblast is the common cell type in the melanomas of all animals, including those of man. The melanoblasts taken from melanomas of living fish, mouse, and man appear to be almost identical when observed in tissue cultures. Neoplasms developing from melanophores are the commonest type of pigment-cell tumors. Taken all together, the number of red and red-black tumors described in all fishes is only eight. This is exactly the number of cases of red-black tumors that we discovered in a *single* brood of hybrids from a mating of an albino swordtail and a uniquely colored black-spotted, red platyfish. The platyfish was produced through crossing-over of the sex chromosomes (YY) of its male parent whose genotype was (Y)*DrSp*/-(Y)*RtSb;* the female parent was (X)*Drsp*/(X)-*Drsp*. The exceptional male produced was (X)-*Drsp*/(Y)*RtSp*. *Dr* for dorsal red is an allele of *Rt* for ruby-throat; *Sp* for side spotting is an allele of *Sb* for belly spotting. A platyfish male gamete containing *DrSp*, fertilizing an albino swordtail ovum, produced hybrids with melanomas somewhere on the body. One containing *RtSb* produced hybrids with melanomas on the mid-ventral areas. One containing *RtSp* produced hybrids with erythromelanomas of the head. The *RtSp* linkage once attained has been maintained for four generations and erythromelanomas have appeared in their hybrid offspring for four generations. These experiments indicate the importance of new combinations of genes and the spatial relationship of genes to each other in producing a specific effect.

THE ACTION OF HORMONES ON UTERINE AND VAGINAL IMPLANTS IN THE

MOUSE. Raymond G. Gottschalk and Phyllis Swartz (by invitation). (Department of Experimental Medicine, Southwestern Medical School of the University of Texas, Dallas, Tex.)

Excised uterine horns, cervices, and vaginas of immature BalbC mice were implanted in the subcutaneous tissue of mice of the same strain. The proportion of takes and the size attained by the transplants decrease from the horns to the vaginas and to the cervices. The grafts are more successful if they are injected into related females than into related males, but a hormonal influence is difficult to demonstrate if transplants are made into adult unrelated females or into unrelated castrated males which are treated or not with estrogens. The size of the grafts varies considerably and it is only little influenced by estrogens.

Implants of adult uterine horns remain generally smaller than those of immature horns. The influence of estrogens on the production of tumors by methylcholanthrene in grafts of uterine horns is being studied.

THE PRODUCTION OF AGGLUTININS IN RABBIT BY INJECTIONS OF PURIFIED EXTRACTS OF BIOPSIED HODGKIN'S LYMPH NODES. C. G. Grand (introduced by Robert Chambers). (New York University, Washington Square College of Arts and Sciences, New York, N.Y.)

Experiments were made to determine whether the purified suspensions of Hodgkin's lymph nodes possess antigenic properties. Extracts of Hodgkin's lymph nodes were subjected to differential ultracentrifugation ($5{,}900 \times g$). A volume of 0.5 cc. of the final, purified suspension, equivalent to 6.25 mg. of the original tissue, was daily injected intravenously for 5–10 days into normal rabbits. Ten days after the last injection, the blood was removed and centrifuged at 2,500 rpm for 20 minutes. The serum was collected and inactivated by heating at 55° C. for 30 minutes. A volume of 0.5 cc. of the serum was diluted with 1 cc. of physiological saline solution. From this, a series of dilutions, ranging from 1:4 to 1:80, was prepared. To each dilution 0.5 cc. of purified Hodgkin's suspension was added. A sample of each dilution was placed in a depression slide, incubated for 30 minutes at 55° C., and finally placed in the refrigerator (4° C.) for 12 hours. These samples, upon examination under the dark-field microscope, exhibited large and small clumps of agglutinated particles. The degree of agglutination was found to be proportional to the dilution of the original serum. For controls, rabbits were injected with similarly prepared purified suspensions of normal lymph nodes, lymphosarcoma, and lymphoid leukemic nodes. The sera of these rabbits did not produce the agglutination reaction when tested against the originally injected purified suspensions. Agglutination reactions were obtained only when Hodgkin's suspensions were added to sera from rabbits injected with the Hodgkin's suspensions. These experiments indicate that the purified Hodgkin's suspension contains an agent which exhibits antigenic properties.

CULTIVATION OF *p*-DIMETHYLAMINOAZOBENZENE-INDUCED RAT LIVER TUMORS IN FERTILE EGGS. A. E. Gray (by invitation), M. I. Armstrong (by invitation), and A. W. Ham. (Departments of Physics and Anatomy, University of Toronto, Toronto, Can.)

Liver tumors were induced in rats by a diet containing 0.06 per cent *p*-dimethylaminoazobenzene. Twenty-eight tumors so produced were transplanted to either chorioallantoic membranes or yolk sacs, or both, of chick embryos. Eighteen tumors grew and were transferred to other eggs. Six did not survive the second transfer; three, the third transfer; two, the fourth transfer; and two, the fifth transfer. Two survived ten transfers; both grew luxuriantly in yolk sacs. The further transfer of one was discontinued voluntarily; the second tumor gives every indication of growing luxuriantly in yolk sacs as long as its regular transfer is continued. Another tumor, transferred as yet only 4 times, is still growing.

Two of the tumors have manifested pronounced lethal effects on chick embryos. The successful serial cultivation of these requires that the tumor tissue be considerably diluted before being injected into yolk sacs, otherwise enough chick embryos do not survive until the fourteenth day of incubation, when the tumors are harvested.

After four transfers in eggs, one tumor was transferred to both the subcutaneous tissues and the peritoneal cavities of rats. In some rats it grew luxuriantly in both sites. When transferred back to yolk sacs, it continued to flourish and exhibited the same histologic and lethal properties as before.

The histologic features of the tumors will be described.

EFFECT OF METABOLITE ANALOGS ON GROWTH OF TRANSPLANTABLE TUMORS. David M. Greenberg, Alice Jean Irish (by invitation), and Emery M. Gal. (Division of Biochemistry, University of California Medical School, Berkeley, Calif.)

The tumor growth-inhibiting properties of maximal doses of certain chemical analogs of amino acids, vitamins, purines pyrimidines, pteridines, and of certain other compounds of special interest were tested, mainly on Sarcoma 37 and the Gardner lymphosarcoma. Amino acid analogs that gave evidence of tumor inhibition without loss of body weight were phenylserine, 3 amino tyrosine, and DL-γ,β-dihydroxy-α-amino-n-butyric acid. The N-substituted amino acids, ethionine, DL-γ-hydroxy-α-amino butyric acid, DL-α-amino-α-methyl butyric acid, DL-α-amino-n-butyric acid, DL-α-amino-isobutyric acid, DL-α-guanidyl-phenylpropionic acid, DL-α-amino-n-valeric acid and chloromycetin were negative.

The vitamin analogs, triaminopyrimidine, tetra-aminopyrimidine, sodium pantoyltaurine, desthiobiotin, ω-methyl pantothenic acid, and 4,6-di-methyl-5-nitrile-2-pyridone were negative on an adequate diet, whereas 2,4-diamino pteridine, out of the eight pteridines tried, induced about a 40 per cent weight regression of Sarcoma 37 when given intraperitoneally.

In agreement with Kidder and co-workers (*Science*, **109**:511, 1949) guanazolo inhibited growth of carcinoma LC8 but proved inactive on Sarcoma 37 and on the Gardner lymphosarcoma.

Of a series of malononitriles, ethoxymethylene malononitrile caused tumor weight reductions.

STUDIES ON THE INHIBITION OF LIVER CATALASE IN TUMOR-BEARING ANIMALS. Robert E. Greenfield and Alton Meister (introduced by Jesse P. Greenstein). (Biochemistry Section, National Cancer Institute, Bethesda 14, Md.)

It has been postulated that the diminished liver catalase of tumor-bearing animals is due to a humoral factor originating in the tumor. However, intraperitoneal injections of tumor extracts in normal animals failed to affect liver catalase activity (Greenstein, J. P., *J. Nat. Cancer Inst.*, **3**:397, 1943). Recently, it has been reported that injection in mice of a crude material obtained from various human tumors, but not analogous products of normal tissues, depressed liver catalase activity (Nakahara, W., and Fukuoka, F., *Gann*, **40**:45, 1949). An attempt has been made to isolate and concentrate the factor responsible for catalase inhibition. In order to obviate some of the biological variability observed by previous workers, inbred strains of animals and transplanted tumors were employed. Catalase determinations carried out with sodium perborate as a substrate varied only ±10 per cent in control animals of the same age and strain. Intraperitoneal injection of partially purified fractions obtained from sarcoma S37 and a transplanted mammary tumor in young C3H mice produced inhibition of liver catalase varying from 10 to 60 per cent. No inhibition *in vitro* was observed. Procedures have been developed for the concentration of the active factor. Studies on the characterization of this agent and its mode of action will be described.

THE INCORPORATION OF RADIOACTIVE PHOSPHORUS, P^{32}, IN THE NUCLEIC ACIDS OF NORMAL AND PRECANCEROUS LIVERS AND OF LIVER TUMORS. A. Clark Griffin, Lew Cunningham (by invitation), Eugenia L. Brandt (by invitation), and D. W. Kupke (by invitation). (Department of Chemistry, Stanford University, Calif.)

Normal rats, rats fed the carcinogenic azo dye, m′-methyl-*p*-dimethylaminoazobenzene, and rats with liver tumors produced by this carcinogen, were injected intraperitoneally with P^{32}. Animals from each group were sacrificed at 3 hours, 18 hours, and 6 days, and the liver and tumor tissue fractionated into acid-soluble, lipid-soluble, desoxyribonucleic acid, and ribonucleic acid components. Nuclei were prepared from these tissues and fractionated into similar components. Total phosphorus and P^{32} were determined on all fractions, while nitrogen, desoxyribonucleic acid, and ribonucleic acid were determined on appropriate fractions.

Incorporation of the P^{32} was studied to determine the relative rates of phosphorus turnover in desoxyribonucleic acid and also nuclear and cytoplasmic ribonucleic acid of normal, precancerous, and tumor tissues of rat liver. Desoxyribonucleic acid of normal liver exhibited a comparatively slow uptake of the isotope while that of precancerous liver and hepatoma tissue had a greater uptake. Six days after administration of the P^{32}, the content of the isotope in precancerous liver desoxyribonucleic acid was 7 times, and that of tumor tissue was 26 times, that of normal. Ribonucleic acid of the cell nucleus incorporated P^{32} very rapidly initially and appeared to be the most active of all phosphorus fractions studied. The increased uptake of isotope in this fraction in precancerous and malignant tissues indicates that nuclear ribonucleic acid possibly acts as an intermediary in nucleic acid synthesis.

THE DISTRIBUTION OF THYROXINE LABELED WITH RADIO-IODINE IN C3H AND C57 MICE. J. Gross and S. Schwartz (introduced by C. P. Leblond). (Department

of Anatomy, McGill University, Montreal, Canada.)

Physiological amounts of thyroxine labeled with radio-iodine were injected into groups of C57, C3H normal mice, and C3H female mice with mammary tumors. Groups were sacrificed at 2 and 24 hours after injection, and the various organs and the excreta were examined for radioactivity.

It was found that the turnover of thyroxine in the C3H strain was somewhat slower than that in the C57 animals, as was also the completeness of utilization as shown by the radioactivity excreted into the urine.

The mammary gland showed an increasing concentration of radioactivity in the following order: C57, C3H not yet bearing tumors, and C3H tumor-bearing animals. At the later time interval, the concentration of radioactivity in the mammary gland and the tumor was about equivalent. The presence of a tumor did not appear to affect the qualitative pattern of thyroxine metabolism in the C3H strain.

GROWTH OF AN UNDIFFERENTIATED MOUSE CARCINOMA IN THE ALBINO RAT.

B. Vincent Hall (introduced by Austin M. Brues). (University of Illinois, Urbana, and Argonne National Laboratory, Chicago, Ill.)

Greene and others have described the growth of malignant tumors in heterospecific hosts when transplanted into the anterior chamber of the eye, but there are only rare accounts of mammalian tumors that grow vigorously when transplanted to other sites. A rapidly growing, undifferentiated mouse carcinoma, which has been cultured at the University of Illinois, Urbana, for 13 years, and recently at the Argonne National Laboratory has been successfully grown in albino rats. Fragments of this mouse tumor, when transplanted into rat uteri, which have been sensitized so as to develop a decidual reaction, grow readily. The tumor will also grow in the rat uterus without the development of a decidua, if it becomes attached to the mesometrium or other well vascularized peritoneal membranes. Subcutaneous transplants did not grow in adult rats. However, when 3- to 5-week rats were used as hosts, subcutaneous implants rapidly grew in 1 week to tumors with average diameters of 13.7 mm. (range 7–25 mm.). These rapidly growing implants from the mouse to young rats have resulted in the death of rats within 10 days after implantation of the tumor fragment, but in most of the cases rats are able to effect regression of even large "mouse" tumors. While transplants of the tumor taken from rats grow vigorously when transplanted into mice, they do not grow readily when transplanted back into the heterospecific host, the rat.

CLINICAL EVALUATION OF A TUMOR LIPOID ANTIGEN.

George C. Hall (by invitation), H. S. Penn, A. H. Dowdy, and A. W. Bellamy (by invitation). (The surgical Service, Wadsworth Hospital, Veterans Administration Center, and the Department of Radiology, School of Medicine, University of California, Los Angeles 24, Calif.)

This report represents a clinical evaluation of a flocculation reaction when an unsaponifiable fraction obtained from human cancerous livers is used as the antigen.

The sera of 6,400 individuals have been investigated by this method in the following categories: (*a*) 2,000 histopathologically proved malignant neoplasms; (*b*) 820 healthy individuals, both sexes, 20–40 age group and 40–60 age group; (*c*) 1,021 pregnancies; (*d*) 2,457 pathological states other than cancer; and (*e*) 102 sera from individuals in fasting state.

The group of histologically verified malignant neoplasms examined were further divided into nine subgroups depending upon their anatomical classification, i.e., head and neck, pulmonary, gastro-intestinal, genito-urinary, breasts, blood dyscrasia, bone tumor, female pelvic organs, and skin.

A number of unknown sera were checked simultaneously in three separate laboratories with a high degree of reliability.

Other phases of the problem relate to the significance of hypo- and hyper-cholesterolemia and the influence of salicylates on the validity of the reaction. Our study at present indicates that 2 per cent positive sera reactions would be found in the general population, and approximately 90 per cent positive sera reactions can be detected in histologically proved instances of malignant neoplasms.

THE METABOLIC DEGRADATION OF 1,2,5,6-DIBENZANTHRACENE-9,10-C^{14}. II. 5-HYDROXY-1,2-NAPHTHALIC ANHYDRIDE, A NEW METABOLITE.

Charles Heidelberger and Walter G. Wiest (by invitation). (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

The only metabolite of dibenzanthracene that has been identified with certainty was shown by others to be 4′,8′-dihydroxydibenzanthracene. We have previously demonstrated with the labeled carcinogen that several acidic metabolites are produced in the mouse and account for the larger

amount of the radioactivity present. We have now shown by carrier experiments that these are carboxylic acids, some containing hydroxyl groups, and that about 8 per cent of the acidic radioactivity was derived from carboxyls originating from carbons 9 or 10, proving that oxidation of the central ring had occurred. The nonradioactive compounds that seemed likely possibilities were added to an acidic radioactive fraction obtained from feces of mice following administeration of the labeled carcinogen as an intravenous colloid. The carrier compounds were isolated and purified by extensive recrystallizations and sublimations. The following compounds did not carry radioactivity after purification, and hence are not metabolites: benzoic acid, phthalic acid, benzene-1,2,3-tricarboxylic acid, benzene-1,2,3,4-tetracarboxylic acid, 1-naphthoic acid, 2-naphthoic acid, 1,2-naphthalic anhydride, 5-hydroxyl-1-naphthoic acid, and 5-hydroxy-2-naphthoic acid. The other likely possibility was 5-hydroxy-1,2-naphthalic anhydride, which was synthesized by Dauben and generously made available to us. This carrier compound maintained constant specific activity after five recrystallizations and five sublimations, and the radioactivity followed the same distribution coefficient and chromatographed in a single band. The metabolite amounted to 5–8 per cent of the acidic radioactivity and was found both free and conjugated. Thus, in order for the central ring to be metabolically oxidized, hydroxyl groups must be present in the terminal rings. This metabolite is now being tested for carcinogenicity.

THE EFFECT OF HEPBISUL (HEPTYL ALDEHYDE–SODIUM BISULFITE ADDITION COMPOUND) AND THYROXIN ON WALKER RAT CARCINOMA 256. PETER A. HERBUT (by invitation), WILLIAM H. KRAEMER, and JOHN JACKSEN (by invitation). (Elizabeth Storck Kraemer Memorial Foundation and Department of Pathology, Jefferson Medical College and Hospital, Philadelphia, Pa.)

Hepbisul and natural thyroxin were administered subcutaneously to Sprague-Dawley rats bearing the Walker rat carcinoma 256. Of the 108 animals treated, 27 showed complete regression of the tumors and 12 others showed a favorable histologic response. A salutary effect, therefore, occurred in 39 animals or 36 per cent of the total. The retrogressive changes consisted of degeneration, necrosis, liquefaction, and fibrosis. The chemicals apparently act directly upon the tumor cells and the fibrosis is compensatory and secondary. Hepbisul and synthetic thyroxin resulted in a favorable response in 2 out of 50 animals treated, or a total of 4 per cent. Despite the relatively high doses used, local irritation and ulceration at the point of injection occurred in less than 50 per cent of the animals, while general toxic effects, as manifested by morphologic changes in the liver and kidneys, were not encountered. The presentation will consist of an outline of the technic employed, a more detailed tabulation of the results, and a kodachrome slide demonstration of the gross and histologic changes induced in the tumors.

CARCINOGENIC ACTION OF THE MUSTARDS. W. E. HESTON. (National Cancer Institute, Bethesda 14, Md.)

To add information regarding the possible correlation between carcinogenic and mutagenic activities of compounds, the nitrogen mustard (methyl-bis β-chloroethyl amine hydrochloride) and sulfur mustard (dichloroethyl sulfide) have been tested for carcinogenic action. The production of lung tumors in strain A mice by the intravenous injection of the compound was chosen as probably the most delicate test for carcinogenicity. In the preliminary test, from 2 to 4 injections of 0.001 mg. of the nitrogen mustard hydrochloride dissolved in 0.01 cc. distilled water per gram body weight significantly increased the incidence and average number of lung tumors. This test has been repeated with comparable results. Four injections of 0.25 cc. of a 1:10 dilution of a saturated solution of sulfur mustard in distilled water (0.06 to 0.07 per cent) per animal also significantly increased the incidence and average number of lung tumors. This test is being repeated. The effect of subcutaneous injection of each compound into strain C3H and strain A mice is being investigated.

OBSERVATIONS ON THE EFFECT OF BERYLLIUM ON GROWTH. MAHLON B. HOAGLAND and MARGARET B. HOOD (introduced by Joseph C. Aub). (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Massachusetts General Hospital, Boston, Mass.)

The sarcomagenic and granulomagenic properties of beryllium, its striking inhibitory effect upon the activity of calcium and magnesium-activated phosphatases, and its close chemical relationship to these metal activators make this element important in the study of normal and malignant growth. The work to be reported was aimed at delineating further the growth responses to the element and clarifying the mechanism of these responses. The complexity of the problem warrants the use of any

growth system from which analogies may be drawn with respect to the animal.

Beryllium, in low concentration, markedly accelerates the growth of plants when it is administered in nutrient solutions which are deficient in magnesium. There is an optimum concentration for this action of beryllium, and somewhat higher concentrations are quite toxic. It has been shown, furthermore, that the glycerophosphatase activity of these plants is strongly inhibited by beryllium, similar to the findings in animal tissues. Beryllium is found not to substitute for magnesium in the chlorophyll of these plants. At present, we are using analogous experimental conditions in animals.

The importance of phosphate compounds in energy metabolism of the plant is, in many respects, similar to that in the animal. The present findings serve to emphasize the fact that beryllium does have growth-stimulating properties under conditions involving alterations in magnesium nutrition and give us a convenient system with which to study the mechanism of these effects.

EFFECTS OF IONIZING RADIATIONS ON A TRANSPLANTED LYMPHOSARCOMA.

JOANNE HOLLCROFT (by invitation), EGON LORENZ, and HARRIET HUNSTIGER (by invitation). (National Cancer Institute, Bethesda 14, Md.)

The lymphosarcoma used in these experiments originated in an irradiated strain A mouse. This tumor when subcutaneously or intramuscularly transplanted remains localized at the site of transplantation and kills the animals in 30–40 days. The whole body irradiations were carried out with x-rays or intravenously injected radon in equilibrium with its short-lived decay products. The radon dose was such that the number of rep (roentgens equivalent physical) given equaled the x-ray dose in roentgens. The following results were obtained: Whole body irradiation with various doses causes regression of the subcutaneously transplanted tumor, dependent upon dose, and there is little difference in the action of both agents upon the tumor. With doses of 400 r or 400 rep, respectively, a few per cent of cures are obtained. For intramuscularly transplanted tumors, the radon treatment proved superior to the x-ray treatment, and approximately 25 per cent cures were obtained. Data on tumor regression and tumor regrowth will be given as well as data on the picture of the peripheral blood. The experimental findings support the possibility that a systemic effect is involved in the regression of the tumor.

SOME OBSERVATIONS IN CANCER PATIENTS RECEIVING ACTH (ADRENOCORTICOTROPIC HORMONE OF THE PITUITARY).

F. HOMBURGER, C. D. BONNER (by invitation), and W. H. FISHMAN. (Clinical Research Laboratories of the Jewish Memorial Hospital, Roxbury, and the Cancer Research and Cancer Control Unit, Departments of Surgery, Medicine, and Biochemistry, Tufts College Medical School, Boston, Mass.)

Owing to the considerable evidence of adrenal dysfunction in patients with cancer, study of the effects of the adrenocorticotropic hormone of the anterior lobe of the pituitary gland (ACTH) on such patients is of interest.

The responsiveness of the adrenal cortex of patients with noncancerous debilitating diseases and of patients with cancer was studied by means of the eosinophile and urinary uric acid response described by Thorn. Atypical response patterns were found in both groups, regardless of the dosage of ACTH. This was not specific for cancer. The response to different preparations of ACTH was evaluated and found to vary considerably.

Changes of blood protein and certain enzyme systems were studied, and some of these were different in the cancer group. The presence of cancer altered the changes of blood sugar caused by ACTH in diabetes.

Results of long-term treatment of some cancer patients with ACTH will be discussed.

EXPERIMENTAL CELLULAR ALTERATION AND DECREASED GROWTH IN A TRANSPLANTED TUMOR.

CHARLES W. HOOKER. (Department of Anatomy, University of North Carolina, Chapel Hill, N.C.)

A testicular tumor composed of primitive Leydig cells that arose in a mouse of the Strong C strain has been carried through 62 serial subcutaneous transfer generations, in mice of the same strain, without cytological change. In earlier experiments, equine gonadotrophin given the hosts provoked various degrees of differentiation of Leydig cells but did not retard growth of the tumor. In the 56th and 62d transfer generations, a concentrated pregnancy urine gonadotrophin was given in daily amounts of 300–10,000 I.U. daily for periods ranging from 2 to 21 days. Single daily injections were without apparent effect. When the daily amount was divided into three injections, the growth of the tumor was retarded, in some instances conspicuously. In these tumors, a fair degree of differentiation toward mature Leydig cells

occurred, and the number of mitotic figures was reduced.

POSTCASTRATION ADRENAL CHANGES AND THE SUBSEQUENT DEVELOPMENT OF MAMMARY CANCER IN SEVERAL INBRED STOCKS OF MICE AND THEIR HYBRIDS. ROBERT A. HUSEBY and JOHN J. BITTNER. (Division of Cancer Biology, Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.)

Adrenal alterations in both male and female mice following castration have been followed in seven inbred stocks and eight F_1 hybrid crosses. Particular note was taken of the latent period prior to the development of a partially cornified vaginal smear, the histology of the adrenal alteration, and the type of sex steroid secreted. From these studies it appears that on the basis of these criteria there is a wide range of responses in the various stocks of mice but that animals of a given inbred of F_1 hybrid stock respond in a very constant manner to gonad removal.

In certain of the stocks, the resulting adrenal hyperplasia or carcinoma was a very effective source of steroid hormones for mammary carcinogenesis in both male and female mice. In the dilute brown stock, D_8 subline, ovariectomized females are having a higher incidence of mammary cancer than are their virgin controls, while castrate males of the same age have as yet developed no mammary tumors.

A FUNCTIONAL OVARIAN TUMOR OCCURRING SPONTANEOUSLY IN A RAT. RIGOBERTO IGLESIAS (by invitation), WILLIAM H. STERNBERG (by invitation), and ALBERT SEGALOFF. (Departments of Pathology and Medicine, Tulane University of Louisiana, and the Alton Ochsner Medical Foundation, New Orleans, La.)

Spontaneous functional ovarian tumors are rare in the mouse and to our knowledge have not previously been reported in the rat. This tumor, which showed marked estrogenic activity and grew progressively on transplantation, occurred in an adult AXC rat. Vaginal smears for 162 days showed irregular cycles, diestrus smears predominating. At autopsy, a tumor 2 cm. in diameter replaced the left ovary. The right ovary was atrophic. A whole mount of mammary glands was equivalent to that of late pregnancy. There were no metastases.

The neoplasm histologically was a granulosa-cell tumor with an admixture of large luteoma cells. Granulosa cells were arranged in diffuse, trabecular, and folliculoid patterns. Mammary glands and vagina were similar, microscopically, to those of late pregnancy.

Over a third of grafts over three transplant generations have, to date, shown progressive growth. Grafts were made subcutaneously and intrasplenically in both male and female, intact and castrate animals. The transplants were histologically similar to the original tumor, although the number of luteoma cells was reduced somewhat.

Transplants in castrate females produced mammary gland proliferation and, in some, estrous smears after a period of diestrus. Transplants in males produced profound testicular atrophy. Preliminary experiments indicate that the presence of the tumor prevents the rise in pituitary gonadotrophin that follows castration. There was some evidence that hormone production by the tumor was not limited to estrogen. However, a single attempt to demonstrate progesterone secretion by the deciduoma-formation test did not succeed.

CYTOTOXIC STUDIES ON MOUSE MAMMARY CANCER CELLS. DAVID IMAGAWA (by invitation), JOHN J. BITTNER, and JEROME T. SYVERTON. (Department of Bacteriology and Immunology, and the Division of Cancer Biology, University of Minnesota, Minneapolis, Minn.)

Samples of antiserum were prepared by employing as antigen for the hyperimmunization of rabbits and guinea pigs (*a*) nonlactating mammary tissues known to contain the mammary tumor agent, (*b*) nonlactating mammary tissues without the mammary tumor agent, and, (*c*) transplantable mammary tumor tissues known to contain the agent. The tests for cytotoxic effect on mammary cancer cells of the samples of serum were carried out by utilizing a mixture made up of one volume of a 10 per cent cancer cell suspension and 5 volumes of antiserum. Each mixture was kept at room temperature for 3 hours and at 4° C. for 3 hours before it was inoculated into susceptible mice.

The results of these experiments establish for the samples of guinea pig serum representative of the mammary tumor agent a marked inhibitory effect which was absent in normal serum and slightly present in antiserum induced by tissue without the agent. The inhibitory effect was decreased after being kept at 56° C. for 30 minutes and restored by normal guinea pig serum, thereby implicating complement. The samples of rabbit serum, on the other hand, showed consistently a definite inhibitory effect on cancer cells, but the effect of normal rabbit serum was slight in com-

parison with that of the samples of antiserum. Surprisingly, serum harvested from mice bearing transplantable tumors and from normal mice with and without the tumor agent produced no cytotoxic effect on cancer cells.

DAMAGE PRODUCED BY A *PSEUDOMONAS AERUGINOSA* FRACTION IN SARCOMA 37. FRANCIS A. JACOBS (introduced by Edward A. Doisy). (Department of Biochemistry, St. Louis University, St. Louis, Mo.)

Cultures of *Pseudomonas aeruginosa* R grown on semi-synthetic media containing glycerol and fatty acids were shown to contain a fraction capable of inducing damage to Sarcoma 37. Intraperitoneal administration of fractions from both the culture fluid and cell pack of the organism to tumor-bearing mice resulted in macroscopic hemorrhage and necrosis of the tumor tissue. Tumor damage was confirmed microscopically.

Fractionation of the culture fluid showed that the activity was in the water-soluble, alcohol-insoluble fraction which contained polysaccharide. The effects produced on the tumors and the host animals were similar to those produced by other tumor-necrotizing agents of bacterial origin.

The measurement of tumor response to treatment is described.

SERUM ALBUMIN IN HUMAN CANCER. ELWOOD V. JENSEN (by invitation) and CHARLES HUGGINS. (University of Chicago, Chicago, Ill.)

Most humans with cancer have albumin with defective reactive groups. The defect can be spotted by thermal coagulative deficiencies of serum and by decreased binding of small dye anions. No significant differences were found on elementary analysis or on determination of thiol or amino groups between fractionated normal or cancerous serum albumin. The defects involve superficial changes in the albumin molecule.

NUCLEIC ACID PHOSPHORUS IN NORMAL, PREGNANCY STIMULATED, AND CANCEROUS MAMMARY GLANDS OF INBRED MICE. RALPH M. JOHNSON (by invitation), S. ALBERT, and MARJORIE S. COHAN (by invitation). (Richard Cohn Radiobiology Laboratory, Detroit Institute of Cancer Research, Detroit 1, Mich.)

Further studies have been made on the uptake of radiophosphorus and the chemical characteristics of the nucleic acid fraction of mammary glands of normal and pregnant mice and of mammary carcinomas. These extend the preliminary observations reported at the previous meeting of the American Association for Cancer Research.

The desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) fractions of these tissues were analyzed chemically for nitrogen, phosphorus, and pentose sugars and measured for radioactivity at various time intervals after injection of P^{32}.

The accumulation of P^{32} in the DNA fraction was greatest in mammary cancers, next highest in the pregnancy stimulated glands, and lowest in normal glands. On the other hand, in the RNA fraction, the order of accumulation of P^{32} was: stimulated glands highest, tumor next, and normal glands lowest.

The chemically determined RNA phosphorus contents of tumors, stimulated and resting glands, respectively, bore the approximate relation 5:3:1, while the DNA phosphorus contents bore the relation 4:1:1. The nitrogen to phosphorus ratios of the DNA fractions of both stimulated and resting glands were found to exceed that of tumors. Pentose determinations indicated that this difference was probably due to an as yet unidentified nitrogenous component.

When attempts were made to separate the DNA from protein, an alkali-resistant, hot trichloracetic acid-insoluble phosphorus moiety remained with the protein, accompanied by a sugar, which differed qualitatively from the sugar found in the DNA.

DO A TRANSPLANTABLE TUMOR AND THE RED BLOOD CELLS OF AN INBRED STRAIN OF MICE HAVE AN AGGLUTINOGEN IN COMMON? NATHAN KALISS and GEORGE E. JAY, JR. (by invitation). (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.)

Inoculations of the transplantable spindle-cell carcinoma 15091a, indigenous to strain A mice, into C57 black mice evokes an antiserum in the latter which will agglutinate strain A red blood cells (Gorer *et al*). Gorer has concluded that tumor and red cells have an antigen in common. However, the agglutinin may have been elicited by the A red cells in the tumor inoculum. We have tried to elucidate this problem by separating tumor 15091a from A red cells.

Tumor 15091a was grafted in unrelated inbred strains (Street, dba-1, dba-2) in which it "takes" in a small per cent of the hosts. Such tumors, now containing "foreign" red cells, were inoculated into C57 black mice. Antisera from the blacks have been tested for agglutinins against A red cells and the "foreign" red cells. Agglutination of A red cells

occurred only with sera from blacks getting their tumors from Street mice. There was no agglutination of "foreign" red cells.

Tumor 15091a was grafted in C57 black mice, previously injected with lyophilized tumor. Grafts grow in most of these mice, whereas they regress in 100 per cent of untreated blacks. These tumors, containing "black" red cells, were inoculated into untreated blacks. Serum from the latter did not agglutinate A red cells.

GROWTH ENHANCEMENT OF TUMOR HOMOIOTRANSPLANTS IN MICE FOLLOWING INJECTIONS OF HOMOGENATES AND ULTRAFILTRATION SEDIMENTS OF MOUSE TISSUES.

NATHAN KALISS, GORDON JONAS (by invitation), and NORMAN L. AVNET (by invitation). (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.)

Prior injections of lyophilized normal mouse tissues will enhance tumor graft growth in an inbred strain of mice in which it normally regresses (Kaliss, N., and Snell, G. D. "Abstr.," *Anat. Rec.*, **105**:3, 1949). The present investigations sought to: (*a*) determine whether injections of fresh tissue homogenates would enhance tumor graft growth and (*b*) localize by ultrafiltration the agent, or agents, for growth enhancement.

Normal liver, kidney, spleen, and tumor 15091a (spindle-cell carcinoma) from strain A (Lilly) mice were the tissues tested. Hosts were C57 black-6 and C57 brown-cd mice. The tumor "takes" in 100 per cent of A (Lilly) mice, and ordinarily regresses in 100 per cent of C57 black and brown mice. Liver and tumor homogenates were filtered under air pressure across cellulose membranes. Groups of the hosts were given a series of intraperitoneal injections of liver and tumor filtrates and sediments, and unfiltered homogenates of liver, kidney, spleen, and tumor. Controls received saline. Fresh tumor was inoculated subcutaneously 1 week after the last injection.

In two experiments, the following numbers, with prior injections as indicated, died with tumor "takes." Experiment 1 (black hosts): liver, 6 out of 19; kidney, 2/19; spleen, 0/4; tumor, 0/20; control, 0/18. Experiment 2: tumor filtrate, 0/11 black mice; tumor sediment, 9/11 black, 7/10 brown mice; unfiltered tumor, 7/10 black, 5/11 brown; liver filtrate, 0/9 black; liver sediment, 1/11 black, 1/11 brown; unfiltered liver, 1/5 black, 2/4 brown; control, 0/10 black, 0/10 brown.

INFLUENCE OF THYMECTOMY, SPLENECTOMY, AND GONADECTOMY ON INCIDENCE OF RADIATION-INDUCED LYMPHOID TUMORS IN STRAIN C57 BLACK MICE.

HENRY S. KAPLAN. (Department of Radiology, Stanford University School of Medicine, San Francisco 15, Calif.)

Previous work has demonstrated that both male and female mice of this strain are highly susceptible to radiation-induced lymphoid tumors, which are prone to originate in the thymus.

In the present studies, litter-mate animals were thymectomized, splenectomized, or kept as intact controls and treated with fractionated whole-body roentgen rays at 1–2 months of age. One lymphoid tumor was observed among 23 thymectomized mice (4 per cent); 13 of 23 (57 per cent) splenectomized and 10 of 21 (48 per cent) control irradiated mice developed such tumors. Thus, thymectomy results in a pronounced decrease in the incidence of radiation-induced lymphomas. This result confirms the previous observations of Furth and associates, working with *spontaneous* lymphomas in susceptible strains.

In another experiment, young mice of both sexes were gonadectomized and litter-mates kept as intact controls. All were exposed to fractional whole-body roentgen rays at 1 month of age. Lymphoid tumors occurred in 11 of 14 intact males (78 per cent), 15 of 18 intact females (83 per cent), 19 of 22 castrate males (86 per cent), and 16 of 20 spayed females (80 per cent). The mean induction times were, respectively, 262, 244, 215, and 234 days. Furth, Murphy, and others, working with *spontaneous* lymphoid tumors in other strains, have observed striking modifications in incidence after gonadectomy. The present experiments do not give evidence of a similar influence of gonadectomy on the incidence of radiation-induced lymphomas in the strain studied.

THE GROWTH AND HISTOLOGY OF A VARIETY OF MOUSE TUMORS EXPLANTED TO THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO.

D. A. KARNOFSKY, P. A. PATTERSON (by invitation), and L. P. RIDGWAY (by invitation). (Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

Twenty-nine different types of mouse tumors were explanted to the chorioallantoic membrane of the 8-day-old chick embryo. These tumors were studied as to their growth on continuous passage in the egg, the histological appearance of the egg-grown tumors, and, after egg passage, their transplantability into susceptible mice.

The behavior of these tumors is tentatively clas-

sified as follows: (*a*) the *leukemias* and *lymphomas*, which fail to show active growth, although some of the cells survived for one egg passage (eight tumors); (*b*) tumors which grew very slowly in mice and also in the egg (four tumors, including melanomas and mammary carcinomas); (*c*) tumors which grew at a moderate rate in mice and also in the egg, but the growth rate decreased with successive passages (seven tumors, chiefly mammary carcinomas); (*d*) tumors which grew at the same rate in mice and on continuous passage in the egg (five tumors, sarcomas); (*e*) tumors growing more rapidly in the egg on continuous passage than in the mouse (one tumor); and (*f*) tumors growing more rapidly in the egg than in the mouse on the first passage, and then failing to grow on subsequent passages (four tumors).

Examples will be shown of the use of egg-grown tumors in cancer chemotherapy studies.

SYNERGISTIC ACTION OF ESTROGENIC HORMONE AND X-RAYS IN INDUCING THYMIC LYMPHOSARCOMA OF MICE.

Arthur Kirschbaum. (Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minn.)

Lymphomas can be induced in mice of the Bagg albino and CBA stocks by the administration of appropriate doses of either estrogenic hormone or x-rays. A single dose of 200 r of x-rays is a minimum inducing dose (4 cases in 72 mice); weekly injections of 5 μg. of estradiol dipropionate for 14 weeks represent a threshold dose for this agent (9 cases in 153 mice).

When these threshold lymphomogenic doses of x-rays and estrogen were given simultaneously to Bagg albino and CBA mice, synergistic effects were observed. Of 69 mice on combined treatment (39 dead), 25 had lymphomatosis and in 22 cases the disease was thymic lymphosarcoma. All induced cases appeared earlier than any spontaneous case of lymphomatosis in 164 untreated controls (all mice were 10 weeks of age when experiments began).

The average age of appearance of the induced lymphomas was 288 days. Although 30 per cent of untreated Bagg albino and CBA mice developed spontaneous lymphomatosis, the average age of appearance was 725 and 610 days, respectively, and only one of 52 spontaneous cases was a mediastinal (thymic) lymphosarcoma.

Thymic lymphoid tissue of young mice is relatively sensitive to the lymphomogenic action of certain agents. Spontaneous lymphocytic neoplastic transformation within the thymus occurs only occasionally in the CBA and Bagg albino stocks (one of 164 mice), although it is readily induced.

FRACTIONATED REGIONAL CANCER CHEMOTHERAPY.

Calvin T. Klopp, Jeanne Bateman (by invitation), Neil Berry, Crandall Alford, and Theodore Winship (introduced by Paul K. Smith). (From the Department of Surgery, the George Washington University School of Medicine and the Department of Pathology, Garfield Memorial Hospital, Washington, D.C.)

The nitrogen mustard compounds have certain characteristics: (*a*) an x-ray-like anti-tumor action; (*b*) a very short reactive life; (*c*) selective action on tumor cells as compared to most other adult cell types; and (*d*) toxic effects on the bone marrow cells (the limiting factor in dosage). The mustards have previously been used for total body therapy and have been effective in certain lymphoid tumors which are also responsive to total body irradiation. The mustards have been relatively ineffective in cancers which are also not responsive to total body irradiation. However, many of these same cancers are responsive to fractionated local irradiation therapy. Therefore, a method was devised whereby nitrogen mustard could be employed in a manner similar to local irradiation, namely, by close intra-arterial injection into the tumor area, and by fractionating the dosage over an extended period of time to minimize bone marrow damage. This increases both the direct antitumor action and the relative selectivity on tumor cells as against normal tissue. Experiments performed in dogs and rabbits demonstrate a localized effect of the drug on the tissues supplied by the artery. Clinical experiments have borne out our concept. A similar localized action, together with tumor regression, has been demonstrated in patients with epidermoid cancers which are not usually responsive to intravenous nitrogen mustard.

THE ACTION OF INTRACYTOPLASMIC PHYSIOLOGICAL MEDIA ON SOL-GEL REACTIONS.

M. J. Kopac. (Department of Biology, Washington Square College of Arts and Science, New York University, New York 3, N.Y.)

Among the various criteria used in evaluating intracytoplasmic physiological media are the effects on sol-gel reactions and on cytoplasmic proteins in *Pelomyxa carolinensis*. One of the most satisfactory media (developed by R. Kassel), as

based on recovery from microinjection and by preservation of microscopic inclusions, contains: KCl 0.042M + NaCl 0.017M + $CaCl_2$ 0.0015M + $MnCl_2$ 0.001M. By contrast, injections of sucrose solutions (0.88M) blocked streaming and locomotion, induced disintegration with large coagulated residues, clumping of granules, etc.

Using the multiple microinjection technic—quadruple micromanipulator equipped with two microinjection and drop-retraction units—the action of aqueous media on cytoplasm was compared to the action of innocuous nonaqueous media. Injections of polarized hydrocarbon oils into *Pelomyxa* produced an irregular-shaped mass as if the oil was poured into a semi-solid mold, i.e., the plasmagel. Drop-retraction revealed slight amounts of surface denatured proteins.

Injections of oil, immediately preceded by injections of small volumes of Kassel's medium, produced completely spherical drops, indicating that the plasmagel was converted to a plasmasol. Subsequent drop-retraction indicated slightly larger amounts of surface-denatured proteins than observed on microinjecting oils alone. Thus, this medium induced immediate solation and a slight destructive action presumably of the submicroscopic particulates.

Present investigations include the modification of Kassel's medium with various substances in order to produce less destruction to submicroscopic particulates. Media fortified with protamine are considerably more destructive. On the other hand, media fortified with either ribose or desoxyribose nucleates are less destructive, as indicated by the extent of surface-denatured proteins recovered at the oil-water interface. Solation of plasmagel regions, however, is still produced. The most important task of intracytoplasmic media is to prevent the submicroscopic particulates from releasing proteins that become surface-denatured whenever oil-water interfacial forces are encountered.

STUDIES OF THE MECHANISM OF PULMONARY TUMOR INDUCTION IN MICE WITH URETHAN.

C. D. Larsen. (National Cancer Institute, Bethesda 14, Md.)

Theoretically, the metabolism of urethan in the body could proceed by either of two pathways, or both. Spontaneously or enzymatically hydrolyzed urethan would result in the formation of ethanol, carbon dioxide, and ammonia. Administration of alcohol, sodium bicarbonate, ammonium carbonate, or ammonium chloride, in molar concentrations comparable to the optimal oncogenic dose of urethan, failed to increase the incidence or multiplicity of lung tumors in strain A mice. Although improbable, degradation of urethan might result in the formation of cyanic acid. Intraperitoneally-administered potassium cyanate exhibited no carcinogenic effect.

METABOLIC STUDIES OF PATIENTS WITH CARCINOMA OF THE BREAST AND THE EFFECTS OF TESTOSTERONE THERAPY.

Daniel Laszlo; Cyril Schulman (by invitation), Judith Bellin (by invitation), Estelle D. Gottesman (by invitation), and Albert Schilling (by invitation). (Division of Neoplastic Diseases, Montefiore Hospital, New York, N.Y.)

Eight patients with advanced carcinoma of the breast were studied under controlled metabolic conditions. The calcium, phosphorus, and nitrogen metabolism were correlated with the clinical course and blood chemical values.

Various aspects of metastatic breast carcinoma were studied—widespread osteolytic metastases, pulmonary metastases, and hypercalcemia. A relationship was noted between the data obtained and the clinical evaluation of the patients.

The effects of testosterone therapy were observed and correlated with the metabolic data. The hypercalcemic syndrome was characterized by marked hypercalcemia, hyperphosphaturia, negative calcium and phosphorous balances, markedly impaired renal function and azotemia. The mechanism of the hypercalcemic syndrome in breast carcinoma with bone metastases was discussed.

THE EFFECT OF THYMECTOMY ON THE INCIDENCE, LATENT PERIOD, AND TYPE OF LEUKEMIA IN HIGH LEUKEMIA STRAINS OF MICE.

L. W. Law and J. H. Miller (by invitation). (National Cancer Institute, Bethesda 14, Md.)

The effect of total thymectomy at 4 weeks of age has been investigated in strain RIL, in which the thymus is initially and principally involved in the leukemic process, and strains C58 and dba, in which the thymus appears to be involved only secondarily in less than 50 per cent of the mice which were examined at autopsy.

A striking reduction in the incidence of leukemia and an increase in the latent period result following thymectomy in all three strains investigated.

The spontaneous and induced leukemias are all typically lymphoid in origin. The leukemias, particularly in strain C58 which appear in thymectomized mice at a later age are likewise typically lymphoid in origin.

Thymectomy does not influence the induction of skin carcinomas or mammary tumors in strain dba painted with methylcholanthrene.

The results of thymectomy and autologous transplantation of thymus tissue and of transplantation of thymus tissue into intact mice are given.

The possible role of the thymus in the leukemic process is discussed.

RELATIONSHIP BETWEEN THYROID ACTIVITY AND LIVER TUMOR INDUCTION WITH 2-ACETYLAMINOFLUORENE. J. H. LEATHEM and H. B. BARKEN (by invitation). (Bureau of Biological Research and Department of Zoology, Rutgers University, New Brunswick, N.J.)

Induction of liver tumors in the rat by 2-acetylaminofluorene is suppressed by thiouracil. Whether or not this action is due to reduced caloric intake has not been determined but warrants consideration, since thiouracil can reduce food intake and drug action may reflect subnormal food intake. Forty-day-old male rats were fed 0.03 per cent 2-acetylaminofluorene (2AAF) and 0.1 per cent thiouracil in fox chow for 6 and 8 month periods. Groups of rats fed the drugs alone, as well as controls, were pair-fed daily. Adrenal and splenic weights were above normal in 2AAF-fed rats, but thiouracil counteracted this effect. Thyroid hypertrophy, which followed thiouracil feeding, was clearly augmented by the carcinogen. 2AAF, alone, and with thiouracil, increased liver weight, but at 8 months the goiterogen appeared to counteract the enhanced carcinogenic action. Grossly, the livers from eight of eleven rats on 2AAF alone were nodular and cystic, with hepatomas in four, in comparison with thiouracil- and 2AAF-treated rats showing four of fourteen livers with gross changes and only cysts, histologically. After 8 months, six cystic livers and one hepatoma were recorded for eight rats despite thiouracil, but 50 per cent of rats fed 2AAF alone had hepatomas, and other livers were cystic.

THE EFFECT OF X-RAYS ON FROG CARCINOMA, STUDIED BY DIRECT MICROSCOPIC EXAMINATION OF LIVING INTRA-OCULAR TRANSPLANTS. BALDUIN LUCKÉ and H. G. SCHLUMBERGER. (Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.)

The renal adenocarcinoma of the leopard frog in intra-ocular transplants affords an excellent preparation for the direct microscopic study of effects of x-rays upon living neoplastic tissue. These irradiation effects have been investigated with single large doses and with repeated small doses.

Three general types of response were observed: (*a*) Cessation of growth; opacity, granularity, and shrinkage of the neoplastic tubules; progressive diminution in size and ultimate reduction of the neoplastic tissue to a fibro-hyaline mass, or its complete disappearance. These changes occurred rapidly after a single large dose of 6,000 r, or more gradually after a single dose of 4,000 r. With repeated small doses, from 7,000 to 10,000 r were required to effect regression. (*b*) Gradual cessation of growth, and continued regression with marked diminution in size for approximately 4 weeks, followed by renewed and progressively very rapid growth of the neoplastic tissue. Such changes were observed after single doses of 2,000 r. (*c*) No visible effects of irradiation, the implants continuing to grow in a normal manner. This was the case when doses of 200 r were given on alternate days for a total of 3,200 r. The absence of any injury of the irradiated tumors was further demonstrated by successfully retransplanting them.

This method of studying the effects of irradiation by direct microscopic observation has brought clear cut confirmation of two concepts widely accepted in radiology: first, that much larger amounts of irradiation are required to produce a certain effect when given in divided doses than in a single dose; second, that incomplete regression of a neoplasm due to inadequate irradiation may lead to resumption of growth at an unusually rapid rate.

THE GROWTH RATE OF TUMOR TRANSPLANTS IN THE CHEEK POUCH OF THE HAMSTER (*MESOCRICETUS AURATUS*). BRENTON R. LUTZ, GEORGE P. FULTON, DONALD I. PATT, and ALFRED H. HANDLER (introduced by Shields Warren). (Department of Biology, Boston University, Boston 15, Mass.)

Sarcomas induced by intramuscular injection of methylcholanthrene or 9, 10-dimethyl-1, 2-benzanthracene in the hamster have been introduced into the cheek-pouch membranes of other hamsters. Both fresh and frozen tissue from the carcinogen-induced tumors have been used, and similarly from the transplants for subsequent transplantation. The advantages of this site for transplantation are (*a*) the tumors grow freely without physical hindrance, (*b*) the same tumor can be observed frequently at successive stages (measured, photographed), (*c*) the tumor may be subjected to vari-

ous experimental procedures (chemical or physical) with no undesired disturbance of the physiological environment beyond that produced by anesthesia, (*d*) transillumination for microscopic study of early stages of growth and vascularization is practicable, and (*e*) a high per cent (nearly 100) of "takes" is obtained. Transplants have been made semi-microscopically, with implant size 0.5–1.0 mm. in diameter, and microscopically by a cell suspension introduced with a micropipette. After a latent period of 1–3 days, during which the transplant becomes vascularized, growth proceeds rapidly and exponentially so that an original transplant of 0.5 cu. mm. may exceed 100 cu. mm. within 2 weeks and 2.3 cc. in 6 weeks. The form assumed by the growing transplant is either a sphere or prolate spheroid, which facilitates fairly accurate determination of volume by measurement with an ocular micrometer. Growth continues, finally, to completely distend the cheek pouch with tumor, when ulceration occurs.

DIFFERENCE IN RATE OF P^{32} TURNOVER OF DIFFERENT TYPES OF NUCLEI IN RABBIT LIVER.

Alfred Marshak. (New York University College of Medicine, New York, N.Y.)

By differential centrifugation it has been possible to separate different types of nuclei from rabbit liver. Fraction 1 contains 99 per cent spherical nuclei and 1 per cent, or less, elongate nuclei. Fraction 2 contains less than 1 per cent of spherical nuclei and 99 per cent elongated nuclei, and about 0.1 per cent of lobulated nuclei. The latter appear to be nuclei of polymorphonuclear leukocytes. There are at least three morphologically distinct types of elongated nuclei which may be identified as coming from reticulo-endothelial, endothelial, and connective tissue cells. Total P^{31} per nucleus is the same for fractions 1 and 2. After intravenous P^{32} administration, the specific activity of fraction 2 is lower than fraction 1 by 30 per cent.

The experiment is given as an example of a procedure whereby physiological activity of nuclei of tumor and stroma cells may be followed separately.

MICROABSORPTION SPECTROSCOPY OF CELLS: STUDIES WITH A REFLECTING ULTRAVIOLET MICROSCOPE.

Robert C. Mellors. (Sloan-Kettering Institute for Cancer Research, The Memorial Hospital Center, New York, N.Y.)

The purpose of this communication is: (*a*) to describe the use of a spectroscopic method which permits the recording of the visible and the ultraviolet absorption characteristics of cells within a period of a few seconds; (*b*) to illustrate the preliminary application of this method to the study of a variety of normal and abnormal cells; (*c*) to indicate to what extent ultraviolet spectroscopic studies can be made of living cells without injuring them; and (*d*) to present some quantitative microabsorption data, the first, in fact, recorded for living cells in the resting and the dividing states. In particular, consideration is given to the several factors that affect the absorption at 260 mμ, which resides principally in the nucleic acids, and to the possible significance of these factors in cellular division.

STUDIES ON THE FORMATION OF PROTEIN-BOUND DERIVATIVES OF 3,4-BENZPYRENE IN THE SKIN OF MICE.

E. C. Miller. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

The epidermis from the skin of mice treated with benzene solutions of 3,4-benzpyrene contains fluorescent substances bound to the protein fraction and apparently derived from the carcinogen. These derivatives are not found in the proteins from the dermis of the treated skin or from the livers, lungs, or kidneys of these mice. The compounds are released by alkaline hydrolysis in the presence of zinc dust, and benzene will extract one fraction from the alkaline hydrolysate and a second after acidification. Both fractions have a blue fluorescence and are distinguished from 3,4-benzpyrene by certain solubility and fluorescence properties. The combination appears to be chemical, since epidermal protein dissolved in weak alkali and reprecipitated by trichloroacetic acid still contains the derivatives, and the compounds are not removed by prolonged extraction of the protein with hot ethanol, ethyl ether, or benzene.

The maximum level is attained about 12 hours after a single application of a 0.2 per cent solution of 3,4-benzypyrene (approx. 250 μg. over 10 sq. cm.). When the solution is applied daily, the maximum is attained after five applications and is 3 times as high as after one application. The epidermis from mice exposed to direct sunlight for several hours a day between applications contains only 40 per cent as much bound fluorescent material as the epidermis from mice kept in darkness. No bound fluorescent compounds are formed when 3,4-benzpyrene is applied to the skin of dead mice.

THE ROLE OF THYROID FUNCTION AND FOOD INTAKE IN OVARIAN TUMORI-

GENESIS IN INTRASPLENIC OVARIAN GRAFTS. O. J. Miller (by invitation) and W. U. Gardner. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Granulosa-cell tumors, luteomas, tubular adenomas, and mixed-cell tumors have developed in intrasplenic ovarian grafts in castrate mice of all strains studied. Hypersecretion of gonadotrophins may be the tumorigenic agent. The effects of hyper- and hypothyroidism and inanition were studied. These alter the type or amount of gonadotrophin secreted or the end organ response.

Hybrid mice (C57 × CBA) of both sexes were castrated, and intrasplenic ovarian grafts made at 40–80 days of age. Ten mice were immediately started on the experimental diets. Dietary controls were fed fox chow with meat meal (Purina). Animals were rendered hyperthyroid, by adding 0.2 per cent desiccated thyroid (Armour) to this diet, and hypothyroid, by the addition of 0.2 per cent thiouracil. Inanition was induced by caloric restriction to 66 per cent of normal. One group was fed *ad libitum* after 6–9 months caloric restriction. Mice underwent autopsies 11–24 months after intrasplenic transplantation.

The aforementioned types of ovarian tumors developed in 16 of 21 dietary controls, 2 of 20 hyperthyroid and 10 of 21 hypothyroid mice, 4 of 12 in the inanition group and 10 of 14 mice fed *ad libitum* after inanition. Signs of estrogen- and progesterone-production were present in all groups except starved mice. An adenoma of the uterine tube developed in one hyperthyroid mouse. Adrenal cortical hyperplasia was marked in mice fed the control diet and absent or minimal in hyperthyroid mice. Ovarian tumorigenesis and its inhibition parallel the development of adrenal cortical hyperplasia.

THE EFFECT OF INFECTION WITH THE VIRUS OF RUSSIAN ENCEPHALITIS ON DIFFERENT TYPES OF TRANSPLANTABLE MOUSE TUMORS. Alice E. Moore and C. C. Stock. (Sloan-Kettering Institute, New York 21, N.Y.)

Previous work has shown that the transplantable mouse tumor Sarcoma 180 can be completely destroyed in the process of infection with the virus of Russian Far-East encephalitis. At the present time, twelve different transplantable mouse tumors have been studied to determine the ability of this virus to interfere with tumor growth.

Bioassay in tumor-susceptible but virus-immune mice was the method used to determine tumor viability. One, 3, and 7 days following the intraperitoneal injection of virus, the tumors were removed, cut into small pieces, and implanted subcutaneously into the proper strain of mice immunized by intraperitoneal injection of formalinized virus. At the time of bioassay, the amount of virus present in the brain and tumor was determined by intracerebral titration.

The tumors investigated are of three groups: (*a*) those which can be completely destroyed, (*b*) those whose growth is inhibited and which can be partly destroyed, and (*c*) those whose growth is unaffected. In the first group are a methylcholanthrene-induced fibro-sarcoma designated MC 1, the mammary adenocarcinoma EO 771, the sarcoma T 241, the carcinoma 1025 and the Ridgway osteogenic sarcoma. In the second group are a transplantable lymphosarcoma and two strains of leukemia, AK 4 and 9421. In the third group are the Wagner osteogenic sarcoma, the sarcoma Ma 387, three squamous-cell carcinomas, the Karnofsky mammary adenocarcinoma, and the Harding Passey melanoma. The virus appears in considerable quantities, but has no appreciable effect on tumor growth in the doses used.

THE CARCINOGENIC PROPERTIES OF 2,7-DIACETYLAMINOFLUORENE, 2,2′-DIACETYLAMINO-9,9′-BIFLUORYL AND BIFLUORYLIDENE. Harold P. Morris and Celia Dubnik. (National Cancer Institute, Bethesda 14, Md.)

The postulation of Pinck (*Ann. New York Acad. Sc.*, **50**:1–18, 1948) that the CH_2 bridge in 2-acetylaminofluorene is of primary importance *in vivo* in the induction of cancer by this compound has been studied. Fluorene compounds containing the 2,7-diacetylamino, the 2,2′-diacetylamino-9,9′-bifluoryl and the 2,2′-diacetylamino-9,9′-bifluorylidene groups were synthesized by Weisburger, Weisburger, and Ray (*J. Am. Chem. Soc.*, **14**:488, 1949) and have been fed to female Sprague-Dawley rats for periods exceeding 30 weeks at a level of .025 per cent in a diet of natural foodstuffs, and for a period of 5 weeks, and also at a level of .025 per cent in a synthetic diet. The tumor incidence has been determined in both groups. It appears that more tumors have been induced by 2,7-diacetylaminofluorene than by 2-acetylaminofluorene. None were induced by the fluoryl derivative and a very few by the fluorylidene derivative. Some of the possibilities of the decreased carcinogenicity of these large molecules in the rat will be discussed.

THE REDUCTIVE CLEAVAGE OF 4-DIMETHYLAMINOAZOBENZENE BY RAT

LIVER HOMOGENATES: THE REACTIVATION OF THE ENZYME SYSTEM BY RIBOFLAVIN-ADENINE DINUCLEOTIDE. GERALD C. MUELLER and J. A. MILLER. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

Rat liver homogenates, prepared in 0.01 M potassium phosphate buffer at pH 7.4 and tested in a reaction mixture fortified optimally with glucose-6-phosphate, triphosphopyridine nucleotide, diphosphopyridine nucleotide, and nicotinamide, have been shown to catalyze the reductive cleavage of the hepatic carcinogen, 4-dimethylaminoazobenzene, to the corresponding monophenyl amines. However, a preliminary treatment of these homogenates at 3° C. with gaseous carbon dioxide or dialysis against carbon dioxide-saturated water resulted in a loss of 50–80 per cent of the enzyme activity. Reactivation of the enzyme system effecting the reductive cleavage was obtained only by the addition of riboflavin-adenine dinucleotide. With preparations of the dinucleotide ranging from 10–61 per cent purity, the degree of reactivation of the carbon dioxide-treated homogenates was proportional to the concentration of the dinucleotide. Riboflavin monophosphate was inactive.

LOCALIZATION OF RADIOACTIVE COMPOUNDS IN TUMORS. WILLIAM G. MYERS. (Medical Biophysics, College of Medicine, Ohio State University, Columbus, Ohio.)

Radioactive sulfanilic acid and 2,6-diiodosulfanilic acids labeled with S-35 have been synthesized in millimole lots in 100 per cent and 70 per cent yields, respectively. These compounds have been diazotized and coupled to intermediates of varied structures to form acidic and basic dyes which were purified chromatographically.

About 1 mg. of the radioactive dyes was injected intravenously into C3H, ABC, CFW, and A mice bearing, respectively, C3HBA adenocarcinoma, 15091a spindle-cell mammary carcinoma, Sarcoma 37, and a transplantable lymphosarcoma which remains localized.

The amounts of radioactivity in the transplanted tumors, blood, skeletal muscle, liver, kidney, and spleen were determined at various intervals after the injections. The relative tumor/blood levels of radioactivities on a wet-weight basis (averages of triplicate determinations) were greater than 5 for two or more of the tumors at one or more intervals after the injections, in the case of 18 of the 70 radioactive compounds tested to date. The most favorable ratios (as high as 20) were usually found in Sarcoma 37 at 96 hours after the injections.

HISTOLOGICAL AND CYTOLOGICAL OBSERVATIONS ON HEREDITARY ERYTHROMELANOMAS IN PLATYFISH-SWORDTAIL HYBRIDS. R. F. NIGRELLI, SOPHIE JAKOWSKA (by invitation), and MYRON GORDON. (New York Aquarium, New York Zoological Society, New York, N.Y.)

Erythromelanomas are produced in the head region of *Platypoecilus maculatus* × *Xiphophorus hellerii* hybrids when Rt gene (red) and Sp gene (spotted black) are linked on the same chromosome in the platyfish. Microscopically, the picture is like that found in melanomas with the exception that the main cellular elements are xantho-erythrophores, with macromelanophores present in smaller numbers. The xantho-erythrophores are spindle-shaped, oval or polygonal and sometimes dendritic, measuring 20–90 μ and their nuclei 5–15 μ. In standard histological preparations, the pigment is removed, and the finely granular cytoplasm is grayish. The nucleus, eccentric in position, may contain one or more nucleolus-like bodies and minute chromatin granules. An occasional mitotic figure is found. In older growths, degenerating cells with vacuolated, reticulated or coarsely granular cytoplasm and with one or more spherical or lobulated nuclei are present. The multinucleated cells may represent a syncytium. The tumors are well vascularized. In smaller tumors, the epithelium is slightly thickened but usually sloughed in larger ones. The connective tissue stroma is well developed, and considerable destruction of bone and muscle is evident. In Feulgen preparations, the pigment granules are yellowish, whereas the melanin in the macromelanophores associated with this tumor retains its characteristic color. This reaction differs from the Harding-Passey mouse melanomas in that the melanin in the latter is partially bleached by this process. The nuclei of the xantho-erythrophores are stained weakly in Feulgen material as compared to the nucleated erythrocytes and other cellular elements in the tumor.

EFFECT OF VARIOUS CARCINOGENS ON TESTOSTERONE-INDUCED COMB GROWTH. K. E. PASCHKIS and A. CANTAROW. (Jefferson Medical College, Philadelphia, Pa.)

R. Hertz and W. Tullner have shown that growth of the chick's comb induced by injection of testosterone propionate is retarded by topical administration of methylcholanthrene. We have

tested the following carcinogens for their influence on androgen-induced comb growth in white Leghorn chicks: methylcholanthrene, benzpyrene, *p*-dimethylaminoazobenzene, 2-acetylaminofluorene, aminofluorene. The inhibitory effect described by Hertz and Tullner for methylcholanthrene is confirmed; aminofluorene application also inhibited comb growth significantly. Benzpyrene, *p*-dimethylaminoazobenzene, and 2-acetylaminofluorene failed to induce significant inhibition.

REMISSION IN ACUTE LEUKEMIA INDUCED BY ACTH. O. H. Pearson, L. P. Eliel, and T. R. Talbot, Jr. (introduced by Konrad Dobriner). (The Division of Clinical Investigation, Sloan-Kettering Institute and the Department of Medicine, Memorial Hospital, New York, N.Y.)

Five previously untreated patients with acute leukemia have received ACTH. Remissions have occurred in all five, three of whom were children, and two adults. One of the children had acute lymphatic leukemia; the other four patients, acute granulocytic leukemia. ACTH was given in a dose of 50 mg. daily to children and 100 mg. daily to adults for periods of 24–30 days. All patients showed symptomatic improvement within the first few days of treatment. There was rapid shrinkage in the size of enlarged spleens and livers. Bleeding from the nose, urinary tract, and bowel, and purpura in the skin and mucous membranes ceased within the first few days of treatment. All patients developed a voracious appetite during the first week of therapy.

The first sign of improvement in the hematologic picture was an increase in the maturity of the circulating white blood cells, which occurred on the fourth to the sixth day of treatment. Increase in the number of reticulocytes occurred on the fifth to the eleventh day. Maximum reticulocyte responses of 10–20 per cent were obtained during the second to third week of therapy. Platelets increased on the ninth to the seventeenth day. In the two adults, the initial white cell count was elevated: 28,000 and 92,000. In both patients, the white count fell to subnormal levels within 2–4 days and then rose slowly to normal levels. The three children had subnormal white counts initially, varying from 650 to 3,200. In all patients, the white cell counts rose slowly, reaching normal levels after 3–4 weeks of therapy.

Improvement in the bone marrow was first manifest by an increase in erythropoietic activity, occurring on the fourth to the eighteenth day of treatment. Increased maturity of the myeloid cells occurred on the eleventh to the 21st day. In none of the patients has there been a complete disappearance of abnormal cells from the bone marrow or peripheral blood.

One child remains in remission 5 weeks after stopping ACTH. Another child developed clinical and hematologic relapse 3 weeks after stopping ACTH. ACTH was again administered with symptomatic improvement. This patient is still receiving treatment. The other three patients continue in remission 1–16 days after discontinuing therapy.

Two children with acute leukemia previously treated with anti-folic substances died 2 and 15 days after beginning ACTH therapy. Death in the patient who received ACTH for only 48 hours was caused by secondary infection as a contributing factor to the leukemia. The second child was critically ill at the time of starting ACTH, with generalized edema, pyrexia and bleeding from the nose, gastrointestinal and urinary tracts. There was some clinical improvement during ACTH therapy as manifested by disappearance of fever and some increase in appetite, but there was no evidence of hematologic improvement.

These studies indicate that remissions can be induced in acute leukemia of both children and adults with considerable regularity by the use of ACTH. At the present state of this study, it must be considered that these remissions are both incomplete and temporary.

TUMOR LIPOIDS AS ANTIGENS (PRELIMINARY REPORT). H. S. Penn. (From the Department of Zoology, University of California, Berkeley, Calif.)

This communication deals with the isolation of lipoid fractions obtained from cancerous livers and tumor-bearing tissue. It has been found that the addition of such fractions to the serum of an individual having cancer results in the appearance of a flocculation, accompanied by a clear supernate. Conversely, the addition of similarly prepared fractions from livers of noncancerous individuals to a positive serum gives negative results.

This investigation was based on a hypothesis of a specific antibody formation in response to an endogenous carcinogen.

A brief description of the preparation and the method of application is given.

A flocculation reaction obtained with lipoids (presumably fatty-acid) from the saponifiable fraction will be briefly discussed.

FURTHER EVIDENCE FOR THE ABSENCE OF THE KREBS CONDENSATION IN TUMOR TISSUES: CITRIC ACID CON-

TENT OF NORMAL AND TUMOR TISSUES *IN VIVO* FOLLOWING INJECTION OF FLUOROACETATE. Van R. Potter and Harris Busch (by invitation). (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

Buffa and Peters reported in 1949 that the injection of fluoroacetate into rats led to large accumulations of citrate in heart, kidney, and brain. Their technic provided an opportunity to test for the occurrence of the Krebs condensation *in vivo*, to determine whether the earlier conclusions from homogenate studies in this laboratory could be supported by an entirely different approach. Rats were injected with 5 mg. of fluoroacetate per kilogram body weight, sacrificed after 1 hour, and the citrate content of various organs and several tumors (Walker 256, Flexner-Jobling, Jensen, and primary hepatoma) was determined. The normal tissues, including blood, as well as the tumors in the uninjected animals, contained about 50 μg. of citrate per gram on the wet weight basis. In the case of the injected animals, high levels of citrate were found in brain, heart, lung, thymus, kidney, spleen, and pancreas (212, 632, 285, 525, 1029, 652, 275 μg. per gram, respectively). Smaller increases were noted in muscle and testis, while no increases were found in liver, blood, or in the tumor samples.

The high levels of citrate in certain organs apparently does not equilibrate with the blood enough to raise its citrate content. The values observed in liver are related to acetoacetate formation in that organ, which can produce large amounts of citrate under certain conditions, in contrast to the tumor tissues, in which there appears to be a real deficiency in the enzymes leading to citrate formation.

A COMPARISON OF THE INTRACELLULAR COMPOSITION OF REGENERATING LIVERS AND INDUCED LIVER TUMORS. J. M. Price and A. K. Laird (introduced by J. A. Miller). (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

Homogenates of regenerating rat livers were separated by differential centrifugation into nuclear, large granule, small granule, and supernatant fluid fractions. The homogenates and fractions were analyzed for protein nitrogen and nucleic acids. Nuclei in the homogenates were enumerated, and the changes occurring during regeneration were expressed per nucleus (cell). Similar calculations were made from previous data on rat liver tumors induced by 4-dimethylaminoazobenzene.

After partial hepatectomy, the desoxypentosenucleic acid content of the remaining nuclei increased to a maximum in 1 day and returned almost to normal in 4 days. The average tumor and normal liver nuclei contained equal amounts of this nucleic acid.

The pentosenucleic acid contents of the small granule and supernatant fluid fractions of regenerating cells increased to a maximum in 1 day and returned to normal levels in 2 more days. The level of this acid in the nuclear fraction reached a maximum in 3 days.

The protein nitrogen contents of the small granules and nuclei of regenerating cells increased during the first 24 hours. During the next 3 days, the nitrogen contents of the cytoplasmic fractions decreased considerably. The ratio of pentosenucleic acid to protein nitrogen of all fractions was increased during rapid regeneration.

The average tumor cell contained about one-third as much pentosenucleic acid and one-fourth as much protein nitrogen as the average liver cell, and the intracellular distribution of each constituent was different from that in normal or regenerating liver cells.

STUNTING OF PLANT SEEDLINGS BY IRRADIATION. Henry Quastler. (University of Illinois, Urbana, Ill.)

Irradiation of plant seedlings produces stunting. The effect involves not growth completed at the time of irradiation, but only the growth expectancy. The substrate of growth expectancy can be broken up into diverse portions which are distinguished by localization, by latency before utilization for actual growth, and by marked differences in susceptibility to irradiation. It appears that the various portions of "substrate of growth expectancy" represent stages in the process preparing for actual growth; the preparatory stages are the ones most susceptible to radiation. The radiation effect on mitosis turned out to be irrelevant in our material. We have not been able to identify the processes involved in radiation-induced stunting of plant seedlings, but some information about them has been derived from an analysis of responses to irradiations under widely varying conditions.

A TUMOR-INHIBITING AGENT PRODUCED BY *ASPERGILLUS FUMIGATUS*. H. Christine Reilly (by invitation) and C. Chester Stock. (Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

During the past 3½ years a search has been made among materials of natural origin for agents that would affect the growth of tumors. In studies carried out on culture media in which microorganisms have been grown, several strains of *Aspergillus fumigatus* have been found to produce a substance or substances that inhibit the growth of Sarcoma 180 in mice. Preliminary observations on other tumors have been made. The active agent of *A. fumigatus* strain 943 has been isolated by complete saturation of a filtrate of the culture medium with ammonium sulfate, suspension of the resulting precipitate in water, and dialysis; the nondialyzable fraction has been lyophilized. The antitumor substance appears to be protein in nature and is not one of the four antibacterial agents that this fungus is known to produce.

Further purification of the active principle has not resulted in any appreciable decrease in its toxicity for mice.

FURTHER STUDIES ON FREE AMINO ACIDS IN NORMAL AND NEOPLASTIC TISSUES. Eugene Roberts and Sam Frankel (by invitation). (Division of Cancer Research, Department of Anatomy, Washington University Medical School, St. Louis, Mo.)

In previously reported work (*Cancer Research*, 9:645, 1949), patterns of free amino acids determined by two-dimensional paper chromatography were studied in a variety of mouse tumors and normal tissues. It was found that each normal tissue had a distribution of free amino acids characteristic for that tissue, while all of the tumors, regardless of derivation or source, showed very similar patterns. The determinations have now been extended to include more normal and neoplastic mouse tissues, and the previous results have been confirmed. A survey of the livers, kidneys, brains, and submaxillary glands of young C3H mice of both sexes revealed no important sex differences. Changes in free amino acids and in peptides were studied in pieces of mouse liver, muscle, squamous-cell carcinoma, and rhabdomyosarcoma which were allowed to autolyze for periods of 2–6 hours under sterile conditions. Changes in these constituents were also studied in homogenates of the above tissues incubated at pH 7.4 without substrate added or with added glutamic or aspartic acid in O_2 at 38° for 2 hours.

AN ETIOLOGICAL STUDY OF THE CANCERS ARISING FROM THE VIRUS-INDUCED PAPILLOMAS OF DOMESTIC RABBITS. Stanfield Rogers (by invitation), John G. Kidd, and Peyton Rous. (The Rockefeller Institute for Medical Research, and Cornell University Medical College, New York, N.Y.)

Transplantation of the cancers (metastatic tissue) to adult rabbits has rarely succeeded, yet seven out of nine have grown rapidly in newborn mongrels. One, the Vx-7, grew in 20 out of 21 individuals of four litters, and hence was transferred to adults. Succeeding in about 20 per cent at first, it now flourishes in all (9th tumor generation).

Adults carrying the Vx-7 develop a complement-fixing antibody against the papilloma virus and another against an antigen contained in the Vx-2, the only cancer derived from a virus papilloma enduringly propagated until now. This antigen has hitherto been peculiar to the Vx-2, an anaplastic, metastasizing, squamous-cell carcinoma which the Vx-7 much resembles, although distinguishable histologically. The Vx-2, for years inducing immunity against the papilloma virus, no longer does so, a fact whereby its hosts can be readily discriminated serologically from those carrying the Vx-7. Whether the Vx-2 antigen, contained in both growths, is peculiar to the cancers derived from virus papillomas remains to be seen.

Even when the Vx-7 had become huge in sucklings, these seldom possessed antibodies against the Vx-2 antigen or the papilloma virus, and, therefore, it seemed likely that they would furnish neoplastic tissue notably favorable to the demonstration of variants of the latter. Three of the cancers grown in them, including the Vx-7, have repeatedly yielded a virus, out of six tested in such relation. Always, it has been merely the papilloma virus, in minute quantity, producing ordinary papillomas on reinoculation.

ENZYME ACTIVITY CHANGES DURING THE EARLY PHASES OF LIVER REGENERATION IN THE RAT. Otto Rosenthal, Charles S. Rogers (by invitation), and James C. Fahl (by invitation). (Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, Penn.)

Young adult, male Wistar rats, which had been kept for at least 1 week on a protein-rich, semisynthetic diet or for 2 weeks on a corresponding protein-free regime, were subjected to a 70 per cent partial hepatectomy and sacrificed between 12 hours and 8 days thereafter. Postoperatively, they were either fed the preoperative diet or fasted up to 4 days. Liver specimens obtained at operation and at sacrifice were assayed for arginase, alkaline

phosphatase, adenosine pyrophosphatase, rhodanese, and nonspecific cholinesterase.

With the latter three enzymes, activity per unit-mass of liver protein remained constant or decreased slightly during the period of most rapid liver growth under all nutritional conditions. In contrast, in protein-depleted animals, particularly when fasted postoperatively, arginase and phosphatase activity doubled or trebled during the first postoperative day, reaching apparently a maximum at the onset of mitotic activity and declining gradually thereafter to normal levels. Concomitantly with this striking increase in enzyme activity, the initial rate of liver protein regeneration was about twice as high as in protein-fed animals.

Profound changes of enzyme activity have not been previously observed in regenerating liver tissue. Our experiments suggest that such changes are limited to enzymes involved in the animal's general metabolism and that they are related to sudden alterations of the nutritional state. Their connection with increased growth rate may indicate that stimuli to regeneration are transmitted through shifts in the enzyme activity pattern.

JOINT ACTION OF A CARCINOGENIC VIRUS AND A CHEMICAL CARCINOGEN TO INDUCE CANCER. Peyton Rous and Stanfield Rogers (by invitation). (The Rockefeller Institute for Medical Research, New York, N.Y.)

Areas of rabbit skin rendered hyperplastic with turpentine were lightly scarified and covered until healed with a dressing that carried methylcholanthrene (*MC*) or 9:10-dimethyl-1:2 benzanthracene (*9:10*). Other scarified areas on the same animals were inoculated with a Shope virus material producing papillomas that became cancerous infrequently, while still others were both inoculated with the virus and dressed with one of the carcinogenic hydrocarbons. In ten experiments 36 rabbits were so treated.

The areas receiving only the hydrocarbon remained bare of growths, but the virus regularly produced typical papillomatous masses. They often appeared slowly, remained smaller, and were relatively inactive where the hydrocarbon also had been applied; yet, here cancers soon arose, often multiple and metastasizing, whereas they seldom did from the control masses, even months later.

On each animal, one of the normal-looking areas, where the hydrocarbons had been applied alone $3\frac{1}{2}$–5 months before, was repeatedly painted with chloroform, an agent devoid of carcinogenicity but stimulating latent neoplastic cells to produce tumors incidentally to the cutaneous hyperplasia it causes; and, in response, papillomas not infrequently arose where *9. 10* had been put. None appeared on the surrounding painted skin, on the unpainted areas, or on those that had received *MC*. Distinctively different from virus papillomas, they resembled those induced by other chemical carcinogens.

The evidence indicates that the *9:10* acted in its carcinogenic capacity, not as a mere promoting agent, when hastening the appearance of cancers in the virus papillomas; and so, too, with the less powerful methylcholanthrene.

FURTHER TRANSFORMATIONS *IN VITRO* OF MOUSE FIBROBLASTS TO SARCOMATOUS CELLS. Katherine K. Sanford, Wilton R. Earle, Mary M. Becker (by invitation), Edward L. Schilling (by invitation), Emily Duchesne (by invitation), Gwendolyn Likely (by invitation), and Emma Shelton (by invitation). (National Cancer Institute, Bethesda 14, Md.)

Twelve strains of fibroblasts from adipose connective tissue and skeletal muscle of C3H strain mice were started in culture between November, 1943, and August, 1948, and were cultivated in an entirely heterologous medium consisting of dilute horse serum and chick embryo extract. Periodically, groups of these cultures were injected into young C3H strain mice to test their ability to grow tumors.

Of five strains of cells carried in culture more than 1 year, four eventually became able to give rise to sarcoma on injection. The fifth was lost before adequate study could be made of it. The one taking the longest interval required nearly 4 years, while the most rapid alteration occurred in 10 months.

Limited groups of cultures from the strains were treated with 20-methylcholanthrene in concentrations of from 0.22 to 0.00008 μg/ml of culture fluid for 21 days, and cultures from these groups were injected periodically thereafter during intervals up to 1 year. This addition of carcinogen caused no recognizable increase in ability of the cells to produce tumors on injection.

The data obtained confirm the alteration *in vitro* of normal fibroblasts to cells able to give rise to sarcoma on injection and indicate that in this laboratory the transformation is the reproducible response of the C3H strain mouse fibroblast to the experimental conditions used.

CYTOLOGICAL ALTERATIONS IN LYMPHOCYTES PRODUCED BY ESTRO-

GENIC HORMONES. GEORGE A. SANTISTEBAN (by invitation), GOTTLIEB L. SCHEEBELI (by invitation), and THOMAS F. DOUGHERTY. (Department of Anatomy, University of Utah College of Medicine, Salt Lake City, Utah.)

Cytological examination of spleens, thymi, and lymph nodes of CBA mice treated with large amounts of estrone or stilbestrol for periods of 2–10 weeks revealed the presence of lymphocytes having atypical mitotic figures.

Throughout the spleen there was an apparent hyperplasia of the reticular cells, the latter being most noticeable in the secondary nodules. Among groups of small and medium-sized lymphocytes at the periphery of the germinal centers were seen the more atypical mitotic figures. These consist of small lymphocytes with chromosomes closely packed against one side of the nuclear membrane and chromosomes apparently extruding through the membrane into the cytoplasm. This appearance suggests a lag in karyokinesis on the part of one of the daughter nuclei, with a complete lack of cytokinesis, so that a mass of chromosomes extruded into the cytoplasm and failed to break away completely from the other more complete nucleus. Other cells had many chromatin fragments within the nucleus and still others a very densely-staining, knot-like mass of chromosomes suggesting polyploidy or chromosome enlargement.

Similarly altered chromosomes and mitotic figures were observed in the lymphocytes of all of the lymphatic organs with the single exception of the thymus.

Since estrogenic hormones are highly leukemogenic and it is known that 10 weeks of hormone treatment is sufficient to induce leukemia in mice of the CBA strain, it is possible that the chromosomal aberrations produced by estrogens may be related to the development of lymphatic leukemia.

THE INFLUENCE OF RIBOFLAVIN, PYRIDOXINE, INOSITOL, AND PROTEIN DEPLETION-REPLETION UPON CHOLINE DEFICIENCY INDUCED NEOPLASMS. A. E. SCHAEFER (by invitation), D. H. COPELAND, W. D. SALMON, and O. M. HALE (by invitation). (Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala.)

Weanling rats of the AES strain were fed a choline-deficient diet with varying levels of riboflavin, pyridoxine, or inositol. Of a total of 68 choline-deficient rats, 84 per cent had liver cirrhosis and 43 per cent had gross tumors. No liver cirrhosis or tumors were found in 24 control rats receiving 0.2 per cent choline chloride supplement plus varying levels of riboflavin, pyridoxine, or inositol. In the choline-deficient group receiving 1 mg. riboflavin/kg of diet 87.5 per cent had cirrhotic livers and 50 per cent had gross tumors. In those receiving 20 mg. riboflavin/kg of diet 75 per cent had mild cirrhosis; however, no gross tumors were noted. Pyridoxine and inositol did not significantly affect the tumor incidence. The rats receiving the choline-deficient diet without pyridoxine supplementation were markedly subnormal in body weight, but seven of eight rats had cirrhotic livers and three had gross tumors. In a separate experiment, ten adult rats undergoing protein depletion-repletion without choline supplementation died within 47 weeks. All had cirrhotic livers and five had gross tumors. Eight control rats receiving 0.2 per cent choline chloride and undergoing protein depletion-repletion for 63 weeks had normal livers. The histological findings will be presented.

METABOLIC STUDIES OF PATIENTS WITH CARCINOMA OF THE PROSTATE AND THE EFFECTS OF STILBESTROL THERAPY. ALBERT SCHILLING (by invitation), JUDITH BELLIN (by invitation), ESTELLE D. GOTTESMAN (by invitation), and DANIEL LASZLO. (Division of Neoplastic Diseases, Montefiore Hospital, New York, N.Y.)

Five patients with advanced carcinoma of the prostate were studied under controlled metabolic conditions. The calcium, phosphorus, and nitrogen metabolism was corrected with the clinical course and the blood chemical values.

The metabolic pattern of these patients before treatment was compared with the data obtained during stilbestrol therapy.

In contrast to the other patients, one case exhibited a moderately elevated calcium excretion in the urine prior to therapy. In this patient, the first manifestation of the estrogen effect was a decrease in the urinary calcium excretion concomitant with a drop in serum acid phosphatase level, even preceding clinical improvement. A positive calcium balance on a low calcium diet was achieved by estrogen therapy, due to the decline in urinary calcium excretion initially and a decline in fecal calcium excretion in the later phase of treatment.

AN EXPERIMENT IN MASS SCREENING OF A POPULATION FOR CANCER, ACTUAL AND POTENTIAL: FINDINGS AT 10,000 EXAMINATIONS. MILDRED W. S. SCHRAM. (North Carolina State Board of Health.)

The organization of these examining centers and the method of examination used are described. Findings, malignant and benign, are tabulated.

FURTHER STUDIES ON THE EFFECT OF TESTOSTERONE PROPIONATE THERAPY ON THE EXCRETION OF HORMONES IN PATIENTS WITH METASTATIC BREAST CARCINOMA. Albert Segaloff; J. V. Schlosser (by invitation), B. N. Horwitt (by invitation), Douglas Gordon (by invitation), and P. J. Murison (by invitation). (From the Department of Medicine, Tulane University, The Alton Ochsner Medical Foundation, and Charity Hospital, New Orleans, La.)

Forty-eight patients have now been studied during testosterone propionate therapy. This was given intramuscularly, 100 mg. every other day, or 3 times weekly.

Twelve of these patients, or 25 per cent, sustained varying degrees of objective regression of the tumor masses. The others were either stationary or showed progression of the disease while under therapy.

It has been possible to obtain hormonal excretion studies both before and at least once during therapy on 23 of the testosterone-treated patients. In almost all of these patients, there was a distinct rise in the urinary excretion of 17-ketosteroids regardless of the response of the patient. The glycogenic corticoid excretion varied widely, and there appeared to be no significant differences which could be correlated with the response of the patients. The same was true for urinary prolactin. In practically every instance there was the expected drop in urinary gonadotrophic hormone.

The finding which appeared to most consistently correlate with improvement in the patient is a distinct decrease in the urinary creatin-creatinine ratio. However, the scatter in this regard between the various patients precludes the use of this as a prognostic measure.

TUMOR SURVEY WITH METHOD FOR DEMONSTRATING ACTIVE CARBONYL GROUPS. Arnold M. Seligman and Rivka Ashbel (by invitation). (Department of Surgery, Harvard Medical School, and Surgical Research Laboratories, Beth Israel Hospital, Boston, Mass.)

Utilizing a method, previously described by us, for demonstrating active carbonyl groups in lipoid and nonlipoid material of tissues, various tumors were examined. Of the tumors of the nervous system, carbonyl groups were demonstrated in nonlipoid material of two Schwannomas and in the lipoid droplets of three hemangioblastomas. Tumors of adrenal cortex, interstitial cell tumors of the dog, and chorioepitheliomas were found to contain lipoid with active carbonyl groups. These results will be illustrated with colored slides, and their significance will be discussed.

HISTOCHEMICAL DEMONSTRATION OF β-GLUCURONIDASE AND SULFATASE. Arnold M. Seligman, Marvin M. Nachlas (by invitation), and Richard Cohen (by invitation). (Department of Surgery, Harvard Medical School, and Surgical Research Laboratories, Beth Israel Hospital, Boston, Mass.)

Principles involved in developing histochemical methods for these two enzymes will be discussed. The best substrate for glucuronidase was prepared by feeding rabbits 6-bromo-2-naphthol followed by isolation of the brucine salt of the glucuronide from the urine. The distribution of glucuronidase in normal and neoplastic tissue with this substrate and diazotized diorthoanisidine (blue color) will be shown with colored lantern slides. The best substrate for sulfatase was potassium 6-benzoyl-2-naphthyl sulfate. The distribution of sulfatase in the tissues of various species will be shown. Human tissues do not hydrolyze this substrate.

EFFECT OF METHYL-BIS (β-CHLOROETHYL) AMINE ON THE SULFHYDRYL CONTENT OF SERUM. Bernard Shacter and Michael B. Shimkin. (Naval Radiological Defense Laboratory, San Francisco Naval Shipyard; and the Laboratory of Experimental Oncology, School of Medicine, University of California Medical Center, San Francisco, Calif.)

Previous studies have indicated that the sulfhydryl content of serum is reduced in several clinical conditions, including neoplastic disease. Moreover, x-radiation of rats is followed in several days by a decrease in the sulfhydryl content of the plasma. The effect of nitrogen mustard, a radiation-mimetic drug on the serum sulfhydryl content of patients with neoplastic disease, was therefore determined.

Following administration of nitrogen mustard to several patients, there was an initial rapid drop in the sulfhydryl content of serum followed by return to initial base line values. As clinical improvement of the patient was noted, there was a concomitant rise in the level of serum sulfhydryl towards values obtained in normal subjects. Al-

though the mechanism of sulfhydryl changes in blood remains to be established, the increase in sulfhydryl content following therapy may serve as another objective measure of clinical improvement.

THE DEVELOPMENT OF MALIGNANT LYMPHOMA IN SOME OF THE YOUNG RATS SUCKLED BY MOTHERS RECEIVING METHYLCHOLANTHRENE BY STOMACH TUBE ONLY DURING LACTATION PERIOD (PRELIMINARY REPORT). HARRY SHAY, SIDNEY WEINHOUSE (by invitation), MARGOT GRUENSTEIN (by invitation), HALVEY E. MARX (by invitation), and BERNICE FRIEDMAN (by invitation). (Samuel S. Fels Research Institute, Temple University School of Medicine.)

Methylcholanthrene instilled into the stomach of the Wistar rat resulted in the development in very high incidence of adenocarcinoma of the breast. (Shay, H.; Aegerter, E. A.; Gruenstein, M.; and Komarov, S. A. Development of Adenocarcinoma of the Breast in the Wistar Rat following the Gastric Instillation of Methylcholanthrene. *J. Nat. Cancer Inst.*, **10**:255–66, 1949.) Reasoning that a possible preferential concentration of absorbed carcinogen in breast tissue might even result in its excretion in the milk, we instilled methylcholanthrene, 2 mg. in olive oil, daily, into the stomach of the mother during the nursing period. The young were then raised on our colony diet without further treatment.

In one group of 61 offspring two instances of reticulum-cell lymphosarcoma arising in the mesenteric lymph nodes appeared at 307 and 385 days respectively. In a second group of 43 offspring born later, one animal died with lymphatic leukemia at 86 days of age. There was an incidence of malignant lymphoma of 2.9 in the 104 offspring. An unfortunate accident in our animal house practically wiped out our experimental colony overnight and left us with the limited results at this time.

Farris and Yeakel (Farris, E. J., and Yeakel, E. H. Spontaneous and Transplanted Reticulum Cell Sarcomas in Wistar Rats. *Am. J. Path.*, **20**: 773–81, 1944.) reported reticulum-cell sarcoma in 0.9 per cent of 1,000 autopsies in Wistar rats (age 331–555 days). No spontaneous lymphatic leukemia has been recorded in this strain.

Using methylcholanthrene tagged with C^{14} as well as by ultra-violet absorption analysis after the administration of nontagged carcinogen, we obtained additional data which indicate that methylcholanthrene may be transferred to the offspring by the mother's milk.

Details of these experiments will be presented.

A COMPARISON OF THE SKIN TUMORS INDUCED BY REPEATED APPLICATIONS OF CARCINOGENIC HYDROCARBONS AND THOSE INDUCED BY A SINGLE APPLICATION OF A CARCINOGEN FOLLOWED BY REPEATED APPLICATIONS OF CROTON OIL. P. SHUBIK (introduced by W. B. Wartman). (Department of Pathology, Northwestern University, Chicago, Ill.)

Tentative suggestions have been put forward (Mottram, J. C. The Change from Benign to Malignant in Chemically Induced Warts in Mice. *Brit. J. Exper. Path.*, **26**:1–4, 1945) that a smaller number of malignant tumors are induced by the method of carcinogenesis using a single application of a hydrocarbon followed by repeated applications of croton oil, than by applying the carcinogen repeatedly.

A survey is being undertaken of the various types of tumor induced by the two methods of carcinogenesis and is concerned, not only with the number of malignant transformations, but also with the various regression rates, and with the biological behavior of the benign tumors induced.

Groups of mice treated respectively with continued methylcholanthrene or 9,10-dimethyl-1,2-benzanthracene applications have been compared with groups of mice receiving a single application of either of these carcinogens followed by repeated applications of croton oil.

All tumors induced have been charted every 2 weeks and finally subjected to histological examination. From a combination of these observations, tumors have been classified under the following headings: (*a*) vigorously growing papillomas that become malignant; (*b*) vigorously growing papillomas that show no tendency to become malignant within the experimental time limits; (*c*) papillomas that become stationary; (*d*) regressions; and (*e*) malignant tumors that appear to have arisen directly as such and appear macroscopically to have by-passed the papilloma stage.

It has been found that the croton oil method gives rise to a far smaller percentage of malignant tumors, but to a larger incidence of vigorously growing papillomas. The regression rate, too, is increased in the croton oil group.

SENSITIVITY OF CHROMOSOMES TO BREAKAGE BY X-RAYS AND ITS RELATIONSHIP TO THE NUCLEIC ACID

CYCLE IN DIVIDING CELLS. Arnold H. Sparrow (by invitation), Montrose J. Moses (by invitation), and Robert Steele. (Department of Biology, Brookhaven National Laboratory, Upton, L.I., N.Y.)

The number of chromosome breaks produced after irradiation by 50 r of 160-kv x-rays, at various stages of the meiotic nuclear cycles, have been studied in *Trillium erectum*. Sensitivity, as determined by counting the number of chromosome fragments, shows at least a 50-fold change. It is highest at late prophase and first metaphase and lowest at early postmeiotic interphase. Since production of acentric fragments leads to cellular dysfunction and death, it is reasonable to assume that our sensitivity-curve is a measure of the ability of the cell to recover from radiation damage.

The experimental modification of the sensitivity of dividing cells (e.g., tumor cells) to x-ray damage would be facilitated by an understanding of the biochemical systems immediately involved. In hopes of delineating some of the factors which determine either high or low sensitivity of the chromosomes to breakage, and since metabolism of the nucleic acids has been shown to be easily upset by x- and ultra-violet irradiation, an attempt was made to correlate changes in PNA and DNA during meiosis with sensitivity of the chromosomes to breakage. Microchemical analyses of the DNA and PNA content of *Trillium* pollen mother cells (PMC) at different meiotic stages show no significant change in amount of either DNA or PNA per PMC throughout divisions. The significance of these results in terms of changes in nucleic acid concentration, synthesis, "target" size and physical effects will be briefly discussed.

THE REPRODUCIBILITY OF THE IODOACETATE INDEX IN THE HUGGINS' TEST. Douglas H. Sprunt, Warren Kyle (by invitation), and Sara G. Richmond (by invitation). (The Division of Pathology and Bacteriology, University of Tennessee, College of Medicine, Memphis, Tenn.)

We are not concerned, in this paper, with whether or not this test is a good diagnostic test for cancer. We are concerned, however, with its reproducibility and with the errors which appear to be inherent in it.

A number of persons were chosen who had iodoacetate indices from 4 to 14. Sufficient blood was taken from these, while fasting, to make possible protein determinations, in triplicate, and the amount of iodoacetate needed to prevent clotting, in duplicate. If all the tubes clotted, or failed to clot, additional tubes were added. Additional samples of blood were taken from these patients at a later time and the test repeated as described.

The various methods of determining whether or not a clot had developed are discussed. For the purpose of this paper, we are using a lead weight. Experiments are now under way with a specially designed viscosimeter. The results with this will be added to this paper.

These experiments are analyzed statistically for the effect of variation in the protein determination, variation in interpretation of whether or not the serum had clotted, and other unknown factors which may alter the test when repeated at 2-week intervals.

These statistical studies are not yet done, but the experiment appears to show, particularly, that if the protein determinations are done with care and the clot determinations made in the same manner with the same criteria, the test is reproducible.

INFLUENZA (TYPE A) VIRUS AS PULMONARY CARCINOGEN IN MICE. Paul E. Steiner and Clayton G. Loosli (by invitation). (From the Departments of Pathology and of Medicine, University of Chicago, Chicago 37, Ill.)

The etiologic relationship of human influenza to lung cancer has long been debated but not finally determined. The problem has now been studied in mice. Animals of the low spontaneous lung tumor strain C57 black and of the high lung tumor strain A were exposed in a chamber to a mist of influenza-A virus (PR-8 strain). They developed the inflammatory, proliferative, and retrogressive pulmonary changes characteristic of influenza in this species. A great tumor-like growth of epithelium was induced in small bronchi and alveoli, but this did not eventuate in tumor. The subsequent incidence of lung tumors was not greater in these than in unexposed controls. Although the virus was a powerful epithelial growth stimulant, it was not a carcinogen as judged by the incidence of mice with lung tumor, number of tumors per mouse, and length of the tumor induction time.

PROTEIN AND METABOLIC STUDIES IN MULTIPLE MYELOMA. Kurt G. Stern and Daniel Laszlo. (Division of Neoplastic Diseases, Montefiore Hospital, New York, and Department of Chemistry, Polytechnic Institute of Brooklyn, N.Y.)

The anomalous protein of low mobility, previously isolated by electrophoresis from the serum of a myeloma patient (K. G. Stern, D. Laszlo, and J. S. Krakauer, *Cancer Research*, **9**:614, 1949), was

found on further study to possess a molecular weight of approximately 150,000, as computed from sedimentation and diffusion measurements. Paper chromatographic analysis (by Dr. R. J. Block) disclosed differences in amino acid composition between the anomalous myeloma protein, normal human gamma globulin, and Bence-Jones protein. The infrared spectrum of the three types of protein is being studied by Dr. E. R. Blout. The anomalous myeloma protein is not a cryoglobulin. Its solubility is similar to that of gamma globulin, but it differs from gamma globulin by its high order of ultracentrifugal and electrophoretic homogeneity.

The electrophoretic diagram of the patient's serum did not change significantly over a 1-year period of observation. In the ultracentrifuge, the area under the very sharply defined boundary ($s'_{20} = 6.9$ S) of the anomalous protein amounts to over 70 per cent of the total, sedimentable material. This concentration agrees with the electrophoretic analysis of the serum.

Ten out of fourteen myeloma sera studied thus far displayed the "gamma-type" electrophoretic pattern, while one serum was found to belong to the rare "alpha-type." Studies on this patient and his serum are in progress.

The rate of protein synthesis (S_K) and the size of metabolic nitrogen pool (P_K) in the first patient, as estimated with the aid of orally administered N^{15}-labeled glycine (with Dr. D. B. Sprinson), were found to be within normal limits ($S_K = 0.2$; $P_K = 0.46$ gm. N/day/kg body weight). The nitrogen, calcium, and phosphorus metabolism has also been studied in this and another patient, and the data will be presented.

INFLUENCE OF SULFONATED AZO DYES ON MOUSE TUMORS. Kurt Stern. (Mount Sinai Medical Research Foundation, Chicago, and Chicago Medical School, Chicago, Ill.)

Injection of water-soluble sulfonated Sudan IV into rabbits was reported to produce proliferation of Kupffer cells (Cestari, 1940). During the past 3 years, five sulfonated azo dyes were tested in mice: Cloth Red B (2 sulfo groups); Brilliant Scarlet 3 R (3 sulfo groups); Wool Red 40 F (3 sulfo groups); Solantine Red 8 BLN (6 sulfo groups); Erie Fast Rubine B Conc. (6 sulfo groups). Aqueous solutions (0.1–1 per cent) were injected subcutaneously once or twice weekly.

Cloth Red B produced a moderate proliferation of Kupffer cells in C57 black and C3H animals, without significant reticulo-endothelial storage of the dye. Development and growth of spontaneous mammary carcinomas in C3H or of methylcholanthrene-induced sarcomas in C57 blacks were not affected by the compound. In 1 out of 28 C3H, and 1 out of 34 C57 blacks, fibrosarcomas arose at the site of injections after 15 and 16 months, respectively. Wool Red 40 and Solantine Red 8 BLN showed neither reticulo-endothelial storage nor any effect on induced or transplanted sarcomas in C57 blacks. Brilliant Scarlet 3 R showed moderate reticulo-endothelial storage but failed to affect mouse tumors.

Erie Fast Rubine B Conc. was avidly stored by reticulo-endothelial cells. It delayed appearance of methylcholanthrene-induced sarcomas in C57 blacks and of transplanted mammary carcinomas in C3H mice. Weight curves of control and treated animals did not differ significantly. The dye did not influence the appearance of spontaneous mammary carcinomas in C3H mice. Slight, statistically insignificant effects of the dye were noted with transplanted sarcomas in C57 blacks and transplanted melanomas in dba. The significance of the findings is discussed.

METABOLISM OF PYRUVATE IN TUMOR HOMOGENATES. Paul A. Stoesz (by invitation), Charles Heidelberger, and G. A. LePage. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

It has been reported (LePage, G. A., *J. Biol. Chem.*, **176**:1009–20, 1948) that in glycolyzing homogenates of Flexner-Jobling rat carcinoma, pyruvic acid disappears from the pyruvate-lactate pool. The extent of the reaction is the same under both aerobic and anaerobic conditions and proceeds at a rate approximately one-third as great as that of the glycolytic system. The reaction requires ATP and DPN.

Studies with $C^{14}O_2$ and with pyruvic acid labeled with C^{14} in either the carbonyl or carboxyl carbon indicate that the reaction does not involve conversion of pyruvate to oxalacetate and that approximately 5 per cent is decarboxylated. The major amount of the product is not volatile with steam, but a small amount of steam volatile acid, which is not formic acid, is formed: this acid corresponds to approximately one-third the amount of pyruvate decarboxylated.

Further studies are being carried on to identify the products of this reaction.

INFLUENCE OF CYSTEINE ON THE RADIOSENSITIVITY OF WALKER RAT CARCINOMA 256. R. L. Straube, H. M. Patt, D. E. Smith, and E. B. Tyree (intro-

duced by A. M. Brues). (Biological Division, Argonne National Laboratory, Chicago, Ill.)

Previous studies have indicated that cysteine is highly effective in reducing the sensitivity of mice and rats to lethal x-radiation. That cysteine might not afford comparable protection to neoplastic tissues was a possibility of some practical as well as theoretical interest. Rats bearing well-implanted Walker 256 mammary carcinomas were injected with cysteine (950 mg/k, i.v.) immediately before total body x-radiation with 800 r. Results indicated that cysteine partially protected tumor cells from the growth-inhibiting action of the x-rays, since such tumors grew more slowly than did the nonirradiated tumors but not as slowly as the control-irradiated tumors. Tumor growth was inhibited during the first 10 days after irradiation; thereafter, the growth rate was similar in cysteine-irradiated, control-irradiated, and nonirradiated tumors. Huge, ulcerated primary tumor masses with widespread metastases caused death of animals surviving the acute radiation period 4–6 weeks after the exposure. Cysteine protected the host from the lethal effect of the irradiation and partially protected the tumor tissue. More of the cysteine-treated animals survived the irradiation, only to succumb to the tumor later. That these effects were not due to differences in nutrition or to the amount of nutrient material available to the tumor was evident in a second experiment on pair-fed rats. A similar pattern of partial shielding of tumor tissue by cysteine administered before irradiation was evoked under these conditions.

ACTION OF 3-BIS (β-CHLOROETHYL) AMINOMETHYL-4-METHOXY-METHYL-5-HYDROXY-6-METHYL PYRIDINE DIHYDROCHLORIDE, 2,4,6-TRIS (1-AZIRIDYL)-*s*-TRIAZINE AND 5-AMINO-7-HYDROXY-lH-*v*-TRIAZOLO (*d*) PYRIMIDINE ON CARCINOMA, SARCOMA, OSTEOGENIC SARCOMA, LYMPHOSARCOMA, AND MELANOMA IN ANIMALS. Kanematsu Sugiura and C. Chester Stock. (Division of Experimental Chemotherapy, The Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

The effect of 3-bis (β-chloroethyl) aminomethyl-4-methoxy-methyl-5-hydroxy-6-methyl pyridine dihydrochloride (SK 1424), 2,4,6-tris (1-aziridyl)-*s*-triazine (SK 1133) and 5-amino-7-hydroxy-lH-*v*-triazolo (*d*) pyrimidine (8-azaguanine) was studied on the growth of Sarcoma 180, Lewis sarcoma T 241, mammary adenocarcinoma EO 771, Harding-Passey melanoma, Wagner osteogenic sarcoma, Ridgway osteogenic sarcoma, Patterson lymphosarcoma, and Mecca lymphosarcoma in mice and Flexner-Jobling carcinoma, sarcoma R 39, and Walker carcino-sarcoma 256 in rats. In general, the first intraperitoneal injection of compounds was given 1–7 days after tumor transplantation and injection was continued for 7–14 days.

The daily doses of 5 mg/kg of SK 1424 had a destructive effect on rat carcinoma, rat sarcoma, and rat carcino-sarcoma, a moderate inhibitory effect on both mouse osteogenic sarcomas, slight inhibitory effect on Sarcoma 180, mouse adenocarcinoma, and both mouse lymphosarcomas, but no effect on sarcoma T 241 and mouse melanoma. The daily doses of 0.25 mg/kg of SK 1133 had a destructive effect on rat carcinoma, rat sarcoma, and rat carcino-sarcoma, a moderate inhibitory effect on both mouse osteogenic sarcomas, a slight inhibitory effect on Sarcoma 180 but no effect on sarcoma T 241, mouse adenocarcinoma, mouse melanoma, and both mouse lymphosarcomas. The daily doses of 50–100 mg/kg of 8-azaguanine had marked inhibitory effect on mammary adenocarcinoma EO 771 but no effect upon the growth of Sarcoma 180, sarcoma T 241, Harding-Passey melanoma, Wagner osteogenic sarcoma, Patterson lymphosarcoma, and Ridgway lymphosarcoma in mice and Flexner-Jobling carcinoma, sarcoma R 39, and Walker carcino-sarcoma 256 in rats. At these levels, the animals showed little or no signs of a toxic effect of the compounds.

SK 1424 and SK 1133 were used to test a large number of rats bearing well-established tumors (1-week-old tumors, F-J carcinoma, 0.8–1.5 cm. diameters; sarcoma R 39, 1–2 cm. diameters; Walker carcino-sarcoma, 1–2.5 cm. diameters). Seven treatments of daily doses of 5 mg/kg of SK 1424 resulted in complete regression of carcinomas in about 75 per cent of the animals, in regression of about 80 per cent of sarcomas, and in about 40 per cent of carcino-sarcomas. Seven treatments of daily doses of 0.25 mg/kg of SK 1133 resulted in complete regression of almost 100 per cent of carcinomas, in about 90 per cent of sarcomas, and in about 30 per cent of carcino-sarcomas.

THE EFFECT OF CORTISONE AND OTHER STEROIDS ON EXPERIMENTAL TUMORS. Kanematsu Sugiura, C. Chester Stock, Konrad Dobriner, and C. P. Rhoads. (Sloan-Kettering Institute for Cancer Research, New York 21, N.Y.)

The effect of cortisone in influencing lymphatic tumors in the mouse and in man has been reported by others. These studies suggested the desirability of examining cortisone and other steroids against a

spectrum of tumors in order (*a*) to challenge the animal tumor test procedure, particularly that with lymphatic tumors, and (*b*) to determine whether the tumor spectrum studies could provide convenient and economical tests of activity that would establish leads for application to human studies.

An extensive study has been made of the effect of cortisone acetate (compound E) and other steroids on the growth of Sarcoma 180, Lewis sarcoma T 241, mammary adenocarcinoma EO 771, Harding-Passey melanoma, the Wagner and Ridgway osteogenic sarcomas, and the Patterson and Mecca lymphosarcomas in mice and sarcoma R 39, the Flexner-Jobling carcinoma, and the Walker carcino-sarcoma 256 in rats. In general, the compounds were injected subcutaneously starting 1 day after implantation of the tumor, and the injections were continued daily for 1 week.

Cortisone acetate (37.5 mg/kg/ day) in aqueous suspension caused a marked inhibition of rat sarcoma R 39, both mouse lymphosarcomas, and both mouse osteogenic sarcomas. A slight inhibition of mouse sarcoma T 241 but no effect upon Sarcoma 180, adenocarcinoma EO 771, and the melanoma have been observed during the period of test, despite relative weight losses in the treated animals. The quantitative aspects are being worked out, and the influence of related steroids will be reported.

HISTOCHEMICAL STUDY OF EPIDERMAL CARCINOGENESIS IN THE MOUSE.

Lloyd E. Thomas and Lotti M. Steinitz (introduced by E. V. Cowdry). (Department of Biochemistry, School of Medicine, University of Missouri, Columbia, Mo.)

Various studies have shown that the conditions of cell life of the cellular population as a whole change fundamentally during epidermal carcinogenesis. A study of such changes, utilizing histochemical methods specific for certain amino acids, should yield information related to the central question of carcinogenesis. The histochemical methods used in this study make use of color reactions which allow detailed microscopic study of paraffin sections. We have found by using sections of agar blocks containing protein that differences in protein concentration of about 15 per cent can be detected by the differences in color intensities. The most consistent difference observed between normal epidermis and the primary squamous-cell carcinoma (induced by painting with methylcholanthrene) is a considerable increase in cytoplasmic protein-bound arginine in the latter. This increase appears to be greater than can be accounted for by the frequently coincident increase in total protein. It is, therefore, highly probable that the ratio of the arginine-containing protein, i.e., protein of the histone type, to the total protein is greater in the primary squamous-cell carcinoma than in normal epidermis or epidermis made hyperplastic by painting with methylcholanthrene. Further implications of these and other observations will be discussed.

COMPLEMENT FIXATION IN ANIMAL NEOPLASIA. IV. THE IMMEDIATE AND DELAYED ANTICOMPLEMENTARY EFFECTS OF SERUM AND ANTIGEN AND THEIR DEPENDENCE UPON THE HEMOLYSIN CONCENTRATION.

Helen Thornton (by invitation), L. D. Ellerbrook (by invitation), S. W. Lippincott, and C. Fong (by invitation). (National Cancer Institute, Bethesda 14, Md., and the Department of Pathology, School of Medicine, University of Washington, Seattle, Wash.)

The immediate anticomplementary effect of frozen, normal rabbit serum decreases as the serum inactivation temperature is increased from 56° to 65°–70° C. A similar additional anticomplementary effect develops during the 18-hour period of incubation at 5° C. used in complement fixation tests of sera of rabbits bearing the Brown-Pearce carcinoma. The antigen (a saline extract of the neoplasm) has an immediate anticomplementary effect, which is not influenced by the inactivation temperature of the normal or reactive serum with which it is mixed. Its 18-hour anticomplementary effect with normal serum is slightly greater with 61° inactivation than at 56° or 65° C. This antigen effect is inhibited by larger amounts of serum. The 18-hour antigen temperature effect is more marked with reactive sera—the complement requirement usually definitely increases as the inactivation temperature is raised from 56° to 61°C., and it then decreases with higher temperatures.

The temperature-effect curves resulting from the use of suboptimal amounts of hemolysin are somewhat similar but higher in the case of serum and of normal serum plus antigen. With reactive serum plus antigen the curves tend to differ in character. With 56° inactivation, the complement requirement increases rapidly as the amount of hemolysin is decreased, while this effect is progressively less pronounced as the inactivation temperature is increased. Apparently, the optimal concentration of hemolysin depends upon the serum inactivation temperature.

VAGINAL AND MAMMARY SENSITIVITY TO ESTROGEN AS RELATED TO MAMMARY TUMOR INCIDENCE OF MICE. J. J. TRENTIN (introduced by W. U. Gardner). (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

The relation of estrogen sensitivity of the vaginal mucosa to mammary tumor incidence has been studied by others using limited numbers of mouse strains. Of those reported, high tumor strains showed a lower vaginal sensitivity to estrogen. A functional correlation has been suggested on the basis that strains with low vaginal sensitivity might secrete larger amounts of estrogen in order to maintain cyclic reproductive activity.

The present study was undertaken to determine whether such an inverse relationship is of universal occurrence or limited to the few strains studied. The following strains and hybrids were used: C3H, CBA, A (high tumor); C57, JK, N (low tumor); BL, AL (C3H and A respectively, free of milk factor); CC1 (C57 ♀ × CBA ♂); CC2 (CBA ♀ × C57 ♂); AB2 (CBA ♀ × A ♂).

Mammary responsiveness to estrogen was also studied in mice of these strains.

Vaginal assays were performed on groups of approximately twenty mice. Graded doses of estrogen were given and repeated until a fairly smooth dose-response curve was attained. The minimum dose of estradiol benzoate giving full cornification in approximately 50 per cent of the animals varied from .06 to .27 μg.

For mammary assay, groups of approximately twenty male mice received a constant dose of estrogen over a 2-week period. The amount of mammary growth induced was determined as the increase in size of the glands over that in groups of twenty control mice.

No correlation could be established between vaginal or mammary estrogen-sensitivity and mammary tumor incidence.

FACTORS INFLUENCING PRENEOPLASTIC MANIFESTATIONS IN MAMMALIAN THYROID GLANDS. JOHN H. VAN DYKE. (Department of Anatomy, Indiana University School of Medicine, Bloomington, Ind.)

Recent studies indicate that ultimobranchial tissue, an accessory epithelial component of the mammalian thyroid gland, is labile—i.e., not necessarily irreversibly differentiated into thyroid tissue during embryonic development.

As a natural embryonic transplant (from pharyngeal entoderm) it becomes transformed into thyroid-like tissue, persisting, as such, after birth usually only in normally active thyroids. In atrophic or deactivated glands it expresses itself as variable cystic masses lined frequently by stratified squamous epithelium. New-growths, which may be aberrant or resemble normal thyroid tissue, arise directly from this epithelium in both active and atrophic thyroids. They may occur either in response to thyroid hyperplasia or thyrotropin (?), in the absence of normal thyroid function (compensatory phenomenon). Neoplastic cells are one of two types, depending on their origin from basal or suprabasal cell layers in the stratified squamous epithelium. Proliferating ultimobranchial cells often stimulate lymphocytic infiltrations and marked eosinophilia. One cell type apparently divides by amitosis. Cystic epithelium may produce cells which spread and as interfollicular cells contribute ultimately to multiple neoplasms.

Since identical manifestations can invariably be produced experimentally in ultimobranchial tissue of rats—through metaplasia—on a vitamin A deficient diet, and the possibility is suggested that suprabasal cells in cyst epithelium may contribute to thyroid adenomata. These cells produce persisting aberrant thyroid tissue during periods of repair, following acquisition of vitamin A. Experiments in progress include those designed to stimulate such cells.

TRICHOMA: TUMOR OF HAIR ANLAGE. STUART A. WALLACE (by invitation) and BÉLA HALPERT. (Department of Pathology, Baylor University College of Medicine and Laboratories, Veterans Administration Hospital, Houston, Tex.)

Neoplasms are derived either from cells making up the individual or from cells left over in the process of development of the individual. The latter group are designated as neoplasms of anlage origin. These neoplasms mimic the structures of the tissue or organ in the formation of which they were superfluous and remained unused. A familiar neoplasm of anlage origin is the ameloblastoma that imitates the structure of the developing enamel body. Also of anlage origin is the so-called basal-cell tumor, which reveals its origin by mimicking the pattern of hair follicles.

The basal layer of the epidermis serves as the delimiting layer of the epithelium toward the corium and aids in the re-covering of denuded surfaces. Repeated attempts by surface epithelial cells to cover a denuded area of skin do not result in a basal-cell tumor. When cancer develops in such an area of chronic ulceration, it is a squamous-cell carcinoma. A basal-cell tumor does not arise from

the basal layer of the epidermis, nor does it behave like a cancerous growth. It appears only at sites where skin appendages are present and commences as a subepidermal nodule. It is locally invasive, and it does not metastasize to regional lymph nodes or to distant parts. There is no more justification to mark as cancerous a patient with a basal-cell tumor than one with an ameloblastoma. The appropriate designation, therefore, for this type of neoplasm is trichoma, tumor of hair anlage.

IN VIVO EFFECTS OF SOME TUMOR-DAMAGING COMPOUNDS ON SEVERAL ENZYMATIC ACTIVITIES OF SARCOMA 37. V. S. WARAVDEKAR (by invitation) and J. LEITER. (Chemotherapy Section, National Cancer Institute, Bethesda, Md.)

Subcutaneous injection of alpha-peltatin (20 μg. per gram of body weight) into mice bearing Sarcoma 37 produced marked damage to the tumors. Enzymatic activity was determined in tumor tissue homogenates from animals sacrificed at 2, 4, 6, 8, 24, and 48 hours after injection. Marked reduction of the cytochrome oxidase activity was observed within 6–8 hours; less than 40 per cent of the activity remained, in comparison with tumors from untreated controls. Estimation of dehydropeptidase and of aldolase activity of extracts of the same tumor homogenates showed that more than 80 per cent of these activities was still present at 8 hours. Dehydropeptidase and aldolase activities were not markedly lowered until more than 24 hours had elapsed after injection. By this time, widespread induced necrosis was evident in the tumors. A diastereoisomer of alpha-peltatin, with little tumor-damaging potency, produced only slight changes in these enzyme activities in the tumors following doses 25 times that of the highly potent alpha-peltatin.

Analogous determinations were carried out in tumors from mice treated with colchicine derivatives with similar findings.

DELAY OF METHYLCHOLANTHRENE CARCINOGENESIS BY 1,2,5,6-DIBENZOFLUORENE. WILLIAM B. WARTMAN; PHILIPPE SHUBIK (by invitation), WILLARD T. HILL (by invitation), B. B. REEB (by invitation), D. WARREN STANGER (by invitation), and BYRON RIEGEL. (Departments of Chemistry and Pathology, Northwestern University, Evanston and Chicago, Ill.)

The addition of an equal amount of 1,2,5,6-dibenzofluorene to methylcholanthrene caused a delay in the latent period of tumor production. CF1 strain mice (Carworth Farms) painted with an acetone solution of this mixture developed tumors 7 weeks later than mice painted with methylcholanthrene alone. Similar experiments with chrysene and methylcholanthrene showed no such effect. The final yield of tumors was the same in both experimental and control groups. These results could not be attributed to differences in the general health of the animals. These results partially confirm those of Lacassagne, A., Buu-Hoi, and Rudali, G. (*Brit. J. Exper. Path.*, **26**:5, 1945).

THE DISTRIBUTION OF 2-ACETYLAMINO-9-C^{14}-FLUORENE AND ω-C^{14}-2-ACETYLAMINOFLUORENE IN THE RAT. ELIZABETH K. WEISBURGER (by invitation), JOHN H. WEISBURGER (by invitation), and HAROLD P. MORRIS. (National Cancer Institute, Bethesda 14, Md.)

The radioactive carcinogen, 2-acetylamino-9-C^{14}-fluorene, in propylene glycol solution was administered by stomach tube to rats. The animals were kept in a metabolism cage for the desired length of time and the excretory products collected. After sacrificing the animal, it was dissected. Aliquots of the organs were assayed by the Van Slyke-Folch method, and the radioactive carbon content determined. The results showed that the highest concentration of radioactivity was in the stomach and intestinal tract. A considerable amount was found in the urine. Other organs which contained appreciable amounts were the kidneys, Harderian gland, liver, lung, brain, pancreas, spleen, heart, blood, and bones. The amount of radioactivity in the expired CO_2 was within the limits of experimental error.

The same procedure was used for studying the distribution of ω-C^{14}-2-acetylaminofluorene (the methyl carbon on the acetyl group was labeled). The greatest amounts were in the small intestine, stomach, and urine. Sizable quantities were in the Harderian gland, liver, brain, kidney, salivary glands, bladder, pancreas, lung, uterus, spleen, and respiratory CO_2. The remaining organs contained small but significant amounts. This preliminary survey indicates that the acetyl group is split off from the acetylaminofluorene.

FURTHER OBSERVATIONS ON COLOR REACTIONS WITH MALIGNANT SERA. EMIL WEISS. (Department of Pathology, People's Hospital, Chicago, Ill.)

A modification of the original procedure of color reactions with malignant sera has been devised. It is a combination of a color reaction and a precipi-

tation reaction. Three dyes are used: Indolin blue, Nicholson's blue, and Sudan black. The dyes are dissolved in isopropyl alcohol in a ratio of 1:1,000. For the test the dyes are further diluted 1:20. A 35 per cent solution of isopropyl alcohol containing a small amount of lipoids extracted from dried beef heart (½ mg. of beef heart to 1 cc. of 35 per cent alcohol kept 2 days at room temperature and then filtered) serves as diluent. The blood should be taken before breakfast. Turbid, hemolytic or icteric sera should not be used. Each unknown requires three tubes, one for each dye. The first tube receives 0.95 cc. Indolin blue, the second tube 0.95 cc. Nicholson's blue, and the third tube 0.95 cc. Sudan black. All three tubes receive 1 drop of the unknown serum. Each dye has a positive and a negative control. The tubes are shaken for a few seconds and then read immediately. A positive tube rapidly becomes turbid, within 30 minutes flocculation occurs, and after 12 hours a dark-colored sediment and a light supernatant fluid are observed. A negative tube becomes only slightly turbid or opalescent and remains the same throughout the test. The procedure is positive in 90 per cent of malignancy. The test applies to all types of malignancy.

THE EFFECTS *IN VITRO* OF SPECIFIC ANTIBODIES ON THE CELLS OF A TRANSPLANTABLE MOUSE LEUKEMIA. Alvar A. Werder (by invitation), Arthur Kirschbaum, and J. T. Syverton. (Department of Bacteriology and Immunology and Department of Anatomy, University of Minnesota, Minneapolis, Minn.)

Serum samples were obtained from rabbits (*a*) which had been hyperimmunized with splenic cells from mice with transplanted myeloid leukemia, line 15 of the F strain, (*b*) which had been hyperimmunized with normal mouse splenic cells, and (*c*) which received no injections. Samples for one series were kept in contact with leukemic cells for 2 hours. Ten thousand cells suspended in 0.2 ml. of each serum were at this time injected intraperitoneally into normal susceptible mice. In a second series of experiments, the procedure was the same except that serum samples were heated for 30 minutes at 60° C. preceding the addition of leukemic cells.

Unheated leukemic tissue rabbit antiserum acted upon leukemic cells *in vitro* so that their capacity to transmit the disease was completely inhibited, whereas treatment with either antiserum against normal mouse cells or normal rabbit serum resulted only in prolongation in survival time.

Complete inhibition by specific mouse leukemic tissue rabbit antiserum persisted in most cases after heating at 60° C. for 30 minutes, whereas normal rabbit serum or antiserum against normal mouse splenic tissue exhibited only a slight inhibitory effect when treated in the same manner.

The antigenicity of mouse leukemic cells is indicated. Specific antibodies were heat-stable, whereas the partially inhibitory substance present in normal rabbit serum and normal tissue antiserum was heat-labile. Further evidence for the specificity of these antibodies was obtained when after iodination with radioactive iodine they were selectively localized in leukemic tissue.

ENZYME INHIBITORS OF THE SERUM IN RELATION TO NEOPLASTIC DISEASE ACTIVITY. Philip M. West. (School of Medicine, University of California at Los Angeles, Los Angeles, Calif.)

Changes in the balance between serum anti-rennin and anti-chymotrypsin provide, with few exceptions, an accurate, objective guide to the progression or regression of neoplastic growth. The effectiveness of therapy in the whole group of malignant diseases may be followed in this way, just as the activity of prostatic carcinoma is commonly traced by serial acid phosphatase determinations. Correlations have been established between the anti-enzyme pattern and neoplastic activity. The close relationship between these factors and the status of the patient is exemplified in the parallel enzymatic and clinical responses to effective therapeutic agents such as nitrogen mustard, x-ray, or hormones. The method possesses no diagnostic significance, and is applied only in uncomplicated cases of proved malignancy.

THE INFLUENCE OF VARIOUS CONCENTRATIONS OF *p*-DIMETHYLAMINOAZOBENZENE ON THE PRODUCTION OF HEPATIC TUMORS IN RATS. Julius White and Rosemary Hein (by invitation). (National Cancer Institute, National Institutes of Health, Bethesda 14, Md.)

Five groups of rats (litter mates) were placed on the basal 6 per cent casein diet employed in this laboratory. To each group was added respectively the following per cent of *p*-dimethylaminoazobenzene: (*a*) 0.06, (*b*) 0.05, (*c*) 0.04, (*d*) 0.03, and (*e*) 0.02. The animals were allowed to ingest the diets until definite tumors were palpable or until the animal died from natural causes. The incidence of hepatic tumors in the five groups were as follows: (*a*) 96.3, (*b*) 99.2, (*c*) 64, (*d*) 54, and (*e*) 54. It therefore appears that a concentration of no less

than 0.05 per cent *p*-dimethylaminoazobenzene should be employed when studying the effects of certain dietary constituents on hepatic tumor formation.

THE INFLUENCE OF CERTAIN DIETS ON THE FORMATION OF HEPATOMAS IN RATS.

JULIUS WHITE, ROSEMARY HEIN (by invitation), and FLORENCE R. WHITE (by invitation). (National Cancer Institute, National Institutes of Health, Bethesda 14, Md.)

Five groups of rats (litter mates) were placed on each of the following diets containing 0.06 per cent *p*-dimethylaminoazobenzene: (*a*) rice and oil, (*b*) rice, yeast, and oil, (*c*) low protein and cystine, (*d*) 5 per cent fat, and (*e*) 25 per cent fat. A comparative study was made of the rate of hepatic tumor formation over a period of 622 days. The following percentages of hepatic tumors were found in each group: (*a*) 60, (*b*) 50, (*c*) 94, (*d*) 94, and (*e*) 88. The significance of these results will be discussed.

VISUALIZATION OF THE PROGRESS OF LIVER INJURY AND THE DEVELOPMENT OF HEPATOMA DURING TREATMENT WITH CARBON TETRACHLORIDE OR AZO DYES IN MICE BY THE USE OF THOROTRAST.

J. W. WILSON, E. H. LEDUC (by invitation), and J. A. CORNER (by invitation). (Department of Biology, Brown University, Providence 12, R.I.)

In mice which have been given Thorotrast (colloidal thorium dioxide) by injection of 0.2 cc. in the tail vein every other day for 3 days (total dose 0.6 cc.), the liver and spleen are rendered opaque to x-rays by the accumulation of the thorium dioxide in the macrophages. This condition remains for a long time (up to 8 months, at least), although the appearance of the liver changes gradually. At first, the liver presents a homogeneous appearance—later it appears spotted or striated. This is shown, histologically, to be due to migration and aggregation of the macrophages.

In carbon tetrachloride injury, involving necrosis of the central third of the lobule, and subsequent repair the macrophages laden with Thorotrast rapidly aggregate around the central veins.

The cirrhosis and carcinogenesis produced by repeated administration of carbon tetrachloride and orthoaminoazotoluene have been followed. The macrophages laden with thorium dioxide tend to accumulate in the fibrotic areas in cirrhosis. They are entirely absent from tumors, which stand out clearly in the x-ray pictures.

Animals in which the presence of hepatomas has been suspected, either after experimental treatment or spontaneously in C3H mice, have been similarly injected and the condition of the liver revealed. Possible harmful effects of Thorotrast will be discussed.

AEROBIC AND ANAEROBIC INCORPORATION OF RADIOACTIVE PHOSPHATE AND RADIOACTIVE GLUCOSE FRAGMENTS BY NORMAL AND CANCER TISSUE *IN VITRO*.

RICHARD J. WINZLER, RICHARD KRAUSE (by invitation), and GRANT G. SLATER (by invitation). (University of Southern California, Los Angeles 7, Calif.)

Cancer tissue exhibits higher aerobic and anaerobic glycolysis rates than most normal stable tissues. It has, therefore, been of interest to compare the rates at which biosynthetic reactions occur in slices of normal and malignant tissues incubated for 4 hours in the presence and absence of oxygen.

The results have indicated that the incorporation of radiophosphate into the protein-bound or lipid fractions of the tumor tissue was less sensitive to anaerobiosis than the incorporation of phosphate into these fractions by the other tissues.

In another series of investigations, the *in vitro* rate of incorporation of photosynthetically prepared radioactive glucose into the lipids and protein-bound fractions of rat sarcoma and rat livers have been investigated in the presence and absence of oxygen. The results have shown that, aerobically, similar amounts of the glucose were oxidized to CO_2 by both tissues. Slight amounts of radioactive CO_2 appeared anaerobically, especially in the tumor tissue.

Less radioactivity was incorporated into the lipids and proteins of both tissues anaerobically than aerobically, the livers being much more affected than the tumor tissue. This was especially true in the lipid fraction.

Current investigations involve a study of the identity of the constituents of the protein-bound fraction into which radioactive glucose fragments are incorporated under aerobic and anaerobic conditions into slices of normal and malignant tissue.

SINUSOIDAL DILATATION OCCURRING IN THE LIVERS OF MICE WITH A TRANSPLANTED TESTICULAR TUMOR.

J. T. WOLSTENHOLME (by invitation) and W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Sinusoidal dilatation occurs in the livers of mice with transplanted granulosa-cell tumors or luteomas. These changes are associated with an increased blood volume and have been described as occurring only with ovarian tumors (Furth and Sobel, 1946; Bali and Furth, 1949).

In the present study of 47 hybrid mice (A strain × C3H strain), sinusoidal changes in the liver similar to those occurring with a granulosa-cell tumor were observed with a transplanted testicular interstitial cell tumor that developed in an estrogen-treated hybrid mouse. The animals studied had tumors derived from the 20th to 33d transfer generation. The livers of the tumor-bearing mice are unusually large. Microscopically, the sinusoids are dilated at the periphery of the hepatic lobule, preserving the central vein. As these changes become more advanced, all of the sinusoids are involved, and the entire lobular architecture of the liver is destroyed. Several of these animals died of hemoperitoneum due to spontaneous rupture of hepatic sinusoids. The most extensive sinusoidal dilatation occurs in mice bearing tumors exceeding 5 gm. at about 4–6 weeks after transplantation.

These tumors showed no estrogenic or androgenic effects, when studied in castrate animals. The present study further dissociates the estrogenic activity and congestive activity of certain tumors.

EFFECT OF HORMONAL SUBSTANCES ON ADRENAL CORTICAL TUMOR FORMATION IN MICE. George W. Woolley. (Division of Steroid Biology, Sloan-Kettering Institute, New York, N.Y.)

Strain CE female mice were gonadectomized at birth and allowed to age without further treatment. This procedure produced adrenal cortical carcinomas in 100 per cent of the mice 6 months of age and older. These tumors were not found in the control strain CE mice. In the present experiments, hormone and hormone-like substances in pellet form were implanted subcutaneously in the gonadectomized mice when 2 months of age. Autopsy was performed when the animals were 6 months of age. Androgens and estrogens prevented tumor formation.

EFFECT OF HODGKIN'S DISEASE BLOOD SERUM ON LYMPHOCYTES OF NORMAL MOUSE LYMPH NODES IN TISSUE CULTURE. Barney Worken (by invitation) and Robert Chambers. (New York University, Washington Square College of Arts and Sciences, New York 3, N.Y.)

The cause of the steady reduction of lymphocytes during the progress of Hodgkin's disease in the involved lymph nodes and spleen is not clear.

It has already been observed in tissue culture studies in this laboratory that when Hodgkin's lymph nodes were grown in a medium containing serum from Hodgkin's patients, the outgrowing lymphocytes undergo early degeneration.

In the present experiments lymph nodes from normal mice were grown in tissue cultures in media containing serum from Hodgkin's patients. These were compared with cultures of similar nodes in normal human serum. The outwandering of many of the lymphocytes from the explants into the plasma clots in cultures with Hodgkin's serum was delayed 12–24 hours. The lymphocytes were sluggish, and many disintegrated within 2–3 days. This was in contrast to the lymphocytes in the control cultures, which were actively motile and remained viable for many days.

The adverse effect on the lymphocytes was observed in 14 of the 22 Hodgkin's sera tested. In the remainder, the lymphocytes did not appear to be affected. There are indications that the observed variability in the effects of the different Hodgkin's sera may be related to the clinical condition of the patient. This aspect is being further studied. Lymphocytes in culture media containing sera from patients with lymphosarcoma, tuberculosis, and a variety of carcinomas and sarcomas were unaffected. In conclusion, a substance toxic to lymphocytes has been found to be present in the blood serum of some cases of Hodgkin's disease.

EFFECT OF A GUANINE ANALOG (GUANAZOLO) ON VIRAL MULTIPLICATION AND ON GROWTH OF THE CHICK EMBRYO. J. S. Youngner and Jonas E. Salk (by invitation). (Virus Research Laboratory, Department of Bacteriology, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.)

Kidder *et al.* reported that growth of the protozoan, *Tetrahymena geleii*, and certain mammalian tumors were inhibited by the guanine analogue, guanazolo, through interference with nucleoprotein synthesis. Since nucleic acid is an important constituent of viruses, experiments were undertaken to determine the effect of guanazolo on virus multiplication.

Mice infected with influenza virus or Lansing-strain of poliomyelitis virus were treated intraperitoneally with daily doses of guanazolo. Mortality was unaltered; in the dosage employed, the drug had no apparent toxic effect in mice.

Preliminary studies with 10-day-old chick em-

bryos showed that in embryos surviving a 48-hour observation period, following inoculation of influenza virus and guanazolo administered via yolk or allantoic sac, growth of the virus was unaffected. However, yolk sac inoculation of the drug induced high mortality rate, hemorrhagic phenomena, and inhibition of growth. Further experiments showed these abnormalities to be due to the action of the guanazolo.

In 4-day-old embryos the influence of guanazolo was more pronounced. A single yolk sac inoculation of 0.125 mg. of the drug produced 80 per cent mortality by the twentieth day of incubation; 0.0625 mg. killed 47 per cent. Dead embryos and many sacrificed at intervals following treatment showed marked inhibition of growth with a high rate of developmental abnormalities. Among the abnormalities were restriction of vascularization in the chorioallantoic membrane, abnormal beaks, generalized edema, exteriorization of viscera, deformed extremities, and hemorrhage. The interfering effect of guanine was studied.

STUDIES ON CROSS-CONNECTIONS BETWEEN CARBOHYDRATE AND PROTEIN METABOLISM IN THE RAT HEPATOMA. Paul C. Zamecnik and Mary L. Stephenson (by invitation). (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Massachusetts General Hospital, Boston, Mass.)

Primary hepatomas were induced in rats by means of a diet containing *p*-dimethylaminoazobenzene. Radioactive glucose, labeled in all 6 carbon positions, was prepared from bean leaves by biological synthesis. *In vitro* experiments were carried out on slices of hepatomas and control adult livers. The tissue slices were incubated in a bicarbonate-buffered, intracellular-type ionic medium, containing the radioactive glucose. In the experiments on hepatoma slices, C^{14} from radioactive glucose was converted into C^{14}-labeled protein at a rate 10–30 times that which occurred in the control liver slices. When 100,000 counts per minute (c.p.m.) of radioactivity was added to hepatoma slices in the form of labeled glucose, approximately 9 times as much radioactivity (800 c.p.m.) was converted into protein as that which was found (90 c.p.m.) when the same number of counts was added with $C^{14}O_2$ as its source. This great difference in the amount of radioactivity fixed into protein depending on source did not occur in the case of the normal liver slice (an average of 46 c.p.m. from C^{14}-glucose versus 39 c.p.m. from $C^{14}O_2$). An interesting possibility would be that 2-carbon or 3-carbon fragments of the glucose molecule are being converted into amino acids in the hepatoma at a rate greater than that which occurs in the normal liver slice.

AUTHOR INDEX

Albert, S., 201, 227
Alford, Crandall, 229
Armstrong, M. I., 201, 221
Ashbel, Rivka, 240
Ashworth, Ethel, 205
Aub, J. C., 207
Avnet, Norman L., 228
Awapara, Jorge, 202

Bang, Frederick B., 219
Barken, H. B., 231
Barnett, D. J., 206
Barton, A. D., 202
Bateman, Jeanne, 229
Bather, Roy, 216
Beck, Lyle V., 202
Becker, Mary M., 238
Bellamy, A. W., 223
Bellin, Judith, 230, 239
Bennett, Warren A., 203
Berger, Ruth E., 204
Berry, Neil, 229
Bierman, Howard R., 203
Biesele, John J., 204
Bittner, John J., 204, 212, 226
Black, Maurice M., 204
Bloom, Walter L., 205
Blumenthal, Herman T., 205
Bonner, C. D., 225
Boutwell, R. K., 202
Brandt, Eugenia L., 222
Breedis, Charles, 205
Broquist, H. P., 208
Broughton, Margaret C., 215
Brues, Austin M., 206
Brunst, V. V., 206
Bryan, W. Ray, 207
Bucher, Nancy L. R., 207
Buckley, S. M., 207
Burchenal, J. H., 208, 209
Burdette, Walter J., 209
Busch, Harris, 236
Byron, R. L., Jr., 203

Cantarow, A., 234
Carruthers, C., 209
Chambers, Robert, 210, 250
Chipps, H. Davis, 210
Chu, C. H. U., 210
Codgell, Betty, 205
Cohan, Marjorie S., 201, 227
Cohen, Richard, 240
Coman, Dale Rex, 212
Copeland, D. H., 211, 239
Corner, J. A., 249
Crossley, M. L., 207, 208
Cunningham, Lew, 211, 222
Curtis, M. R., 213

Danish, Abram, 211
Davidsohn, Israel, 211, 212
DeLong, Robert P., 212
Diller, Irene Corey, 212
Dobriner, Konrad, 213, 244
Dougherty, Thomas F., 239
Dowdy, A. H., 223
Dubnik, Celia, 233
Duboff, Gregory S., 213
Duchesne, Emily, 238
Dunning, W. F., 213
Dyer, Helen M., 214

Earle, Wilton R., 214, 238
Eddie, Bernice U., 203
Eichwald, E. J., 214
Eliel, L. P., 235
Ellerbrook, Lester D., 215, 245
Engel, R. W., 211, 215
Eriksen, Nils, 215

Fahl, James C., 237
Faloon, William W., 215
Figge, Frank H. J., 206
Firminger, Harlan I., 216
Fishman, William H., 216, 225
Fong, C., 245
Frankel, Sam, 237
Franks, W. R., 216, 217
Frantz, Ivan D., Jr., 207
Friedgood, Charles E., 217
Friedman, Bernice, 241
Friedman, Nathan B., 218
Friedman, Orrie M., 218
Fulton, George P., 231

Gal, Emory M., 221
Gardner, Lytt I., 218
Gardner, W. U., 219, 233, 249
Gey, George O., 219
Goldfeder, Anna, 219
Goldsmith, E. D., 220
Gordon, Douglas, 240
Gordon, Myron, 220, 234
Gorham, L. Whittington, 215
Gottschalk, Raymond G., 221
Gottesman, Estelle D., 230, 239
Grand, C. G., 221
Gray, A. E., 221
Green, Morris N., 217
Greenberg, David M., 221
Greenfield, Robert E., 222
Greiff, Donald, 205
Griffin, A. Clark, 211, 222
Gross, J., 222
Gruenstein, Margot, 241

Hale, O. M., 239
Hall, B. Vincent, 223
Hall, George C., 223
Halpert, Béla, 246
Ham, Arthur W., 201, 221
Hammon, William McD., 203
Handler, Alfred H., 231
Harnly, Morris H., 220
Haven, Frances L., 205
Heidelberger, Charles, 223, 243
Hein, Rosemary, 248, 249
Herbut, Peter A., 224
Heston, W. E., 224
Higgins, George M., 203
Hill, Willard T., 247
Hitchings, George H., 204
Hoagland, Mahlon B., 224
Hollcroft, Joanne, 225
Homburger, F., 216, 225
Hood, Margaret B., 224
Hooker, Charles W., 225
Horwitt, B. N., 240
Huggins, Charles, 227
Hunstiger, Harriet, 225
Huseby, Robert A., 226

Iglesias, Rigoberto, 226
Imagawa, David, 226
Irish, Alice Jean, 221

Jacksen, John, 224
Jacobs, Francis A., 227
Jakowska, Sophie, 234
Jay, George E., Jr., 227
Jensen, Elwood V., 227
Johnson, Ralph M., 201, 227
Johnston, S. F., 208
Jonas, Gordon, 228
Jukes, T. H., 208

Kaliss, Nathan, 227, 228
Kaplan, Henry S., 228
Karnofsky, D. A., 228
Kasdon, S. Charles, 216
Kidd, John G., 237
Kirschbaum, Arthur, 229, 248
Kleiner, I. S., 204
Klopp, Calvin T., 211, 229
Kopac, M. J., 229
Kraemer, William H., 224
Kraul, Louis H., 210
Krause, Richard, 249
Kupke, D. W., 222
Kyle, Warren, 242

Laird, A. K., 236
Lanman, Jonathan T., 203
Larsen, C. D., 230
Laszlo, Daniel, 230, 239, 242
Law, L. W., 230
Leathem, J. H., 231
Leduc, E. H., 249
Leiter, J., 247
LePage, G. A., 243
Lewis, George T., 205
Lieberman, Seymour, 213
Likely, Gwendolyn, 238
Lippincott, Stuart W., 215, 245
Loosli, Clayton G., 242
Lorenz, Egon, 207, 225
Luck, J. Murray, 211
Lucké, Balduin, 231
Lutz, Brenton R., 231

Marshak, Alfred, 232
Marx, Halvey E., 241
Maun, M. E., 213
Meek, G. A., 217
Meister, Alton, 222
Mellors, Robert C., 232
Meyer, Karl F., 203
Miller, E. C., 232
Miller, J. A., 234
Miller, J. H., 230
Miller, O. J., 233
Moloney, John B., 207
Moore, Alice E., 233
Morris, Harold P., 214, 233, 247

Moses, Montrose J., 242
Mueller, Gerald C., 234
Murison, P. J., 240
Myers, William G., 234

Nachlas, Marvin M., 240
Nigrelli, R. F., 234

Owens, Lawrence A., 215

Paschkis, K. E., 234
Patt, Donald I., 231
Patt, H. M., 243
Patterson, P. A., 228
Pearson, O. H., 235
Penn, H. S., 223, 235
Potter, Van R., 236
Price, J. M., 236

Quastler, Henry, 236

Reeb, B. B., 247
Reilly, H. Christine, 236
Rhoads, C. P., 207, 208, 209, 213, 244
Richmond, Sara G., 242
Ridgway, L. P., 228
Riegel, Byron, 247
Rietz, Leola, 206
Roberts, Eugene, 237
Rogers, Charles S., 237
Rogers, Stanfield, 237, 238
Rosenthal, Otto, 237
Ross, Helen E., 214
Rous, Peyton, 237, 238
Rusch, Harold P., 202
Rutenberg, Alexander M., 218

Salk, Jonas E., 250
Salmon, W. D., 239
Sanford, Katherine K., 238
Santisteban, George A., 239
Schaefer, A. E., 239
Scheebeli, Gottlieb L., 239
Schilling, Albert, 230, 239
Schilling, Edward, 214, 238
Schlosser, J. V., 240
Schlumberger, H. G., 231
Schram, Mildred W. S., 239
Schulman, Cyril, 230
Schwartz, S., 222
Scott, J. F., 207
Segaloff, Albert, 226, 240
Seligman, Arnold M., 218, 240
Shacter, Bernard, 240
Shay, Harry, 241
Shelton, Emma, 214, 238
Sheremetieva-Brunst, E. A., 206
Shimkin, Michael B., 203, 240
Shubik, P., 241, 247
Slater, Grant G., 249
Smith, D. E., 243
Sniffin, Ronald C., 218
Sparrow, Arnold H., 242
Speer, F. D., 204
Sprunt, Douglas H., 242
Stanger, D. Warren, 247
Steele, Robert, 242
Steiner, Paul E., 242
Steinitz, Lotti M., 245
Stephenson, Mary L., 251
Stern, Kurt, 211, 212, 243
Stern, Kurt G., 242
Sternberg, William H., 226
Stock, C. Chester, 207, 208, 209, 233, 236, 244
Stoesz, Paul A., 243
Straube, R. L., 243
Sugiura, Kanematsu, 244
Suntzeff, V., 209
Swartz, Phyllis, 221
Syverton, Jerome T., 226, 248

Talbot, Nathan B., 218
Talbot, T. R., Jr., 235
Thomas, Lloyd E., 245
Thompson, J. S., 217
Thornton, Helen, 245
Trentin, J. J., 246
Tyree, E. B., 243

Van Dyke, John H., 246

Wallace, Stuart A., 246
Walsh, Lawrence B., 205
Waravdekar, V. S., 247
Wartman, William B., 247
Weinhouse, Sidney, 241
Weisburger, Elizabeth K., 247
Weisburger, John H., 247
Weiss, Emil, 247
Werder, Alvar A., 248
West, Philip M., 248
White, Florence R., 249
White, Julius, 248, 249
Wiest, Walter G., 223
Wilson, Hildegard, 213
Wilson, J. W., 249
Winship, Theodore, 229
Winzler, Richard J., 249
Wolstenholme, J. T., 249
Woods, Kathryn A., 203
Wooley, George W., 250
Worken, Barney, 250

Youngner, J. S., 250

Zamecnik, Paul C., 251
Zygmuntowicz, Aniela S., 218

Announcement

The Special Training Division of the Oak Ridge Institute of Nuclear Studies will conduct the fifteenth, sixteenth, and seventeenth in its series of basic radioisotope technics courses this summer. Dates for the courses are June 5–30, July 3–28, and July 31–August 25. Priority will go to university personnel who have difficulty attending courses offered during the winter months. The courses are open to senior research personnel.

Thirty-two participants will be accepted for each of the three repeated courses. This number is dictated by the laboratory facilities available for use in the courses.

Each session is comprised of laboratory work, lectures on laboratory experiments, general background lectures, and special-topic seminars. Experiments cover the use and calibration of instruments and purification and separation procedures, as well as the application of a number of radioisotope technics. Seminars include such discussions as the use of radioisotopes in animal and human experimentation, principles and practices of health-physics, design of radiochemical laboratories, dosimetry, instrumentation, and radiation effects on cells.

A registration fee of $25.00 is charged for the course. Dormitory or hotel facilities are available in Oak Ridge for participants.

Application forms and additional information on the courses may be obtained from Dr. Ralph T. Overman, Chairman, Special Training Division, Oak Ridge Institute of Nuclear Studies, P.O. Box 117, Oak Ridge, Tennessee.